First trimester serum tests for Down's syndrome screening (Review)

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[Diagnostic Test Accuracy Review]

First trimester serum tests for Down's syndrome screening

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ABSTRACT

Background

Down's syndrome occurs when a person has three, rather than two copies of chromosome 21; or the specific area of chromosome 21 implicated in causing Down's syndrome. It is the commonest congenital cause of mental disability and also leads to numerous metabolic and structural problems. It can be life-threatening, or lead to considerable ill health, although some individuals have only mild problems and can lead relatively normal lives. Having a baby with Down's syndrome is likely to have a significant impact on family life.

Noninvasive screening based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allows estimates of the risk of a pregnancy being affected and provides information to guide decisions about definitive testing. However, no test can predict the severity of problems a person with Down's syndrome will have.

Objectives

The aim of this review was to estimate and compare the accuracy of first trimester serum markers for the detection of Down's syndrome in the antenatal period, both as individual markers and as combinations of markers. Accuracy is described by the proportion of fetuses with Down's syndrome detected by screening before birth (sensitivity or detection rate) and the proportion of women with a low risk (normal) screening test result who subsequently had a baby unaffected by Down's syndrome (specificity).

Search methods

We conducted a sensitive and comprehensive literature search of MEDLINE (1980 to 25 August 2011), Embase (1980 to 25 August 2011), BIOSIS via EDINA (1985 to 25 August 2011), CINAHL via OVID (1982 to 25 August 2011), The Database of Abstracts of Reviews of Effectiveness (*The Cochrane Library* 25 August 2011), MEDION (25 August 2011), The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (25 August 2011), The National Research Register (Archived 2007), Health Services Research Projects in Progress database (25 August 2011). We did forward citation searching ISI citation indices, Google Scholar and PubMed 'related articles'. We did not apply a diagnostic test search filter. We also searched reference lists and published review articles.

Selection criteria

We included studies in which all women from a given population had one or more index test(s) compared to a reference standard (either chromosomal verification or macroscopic postnatal inspection). Both consecutive series and diagnostic case-control study designs were included. Randomised trials where individuals were randomised to different screening strategies and all verified using a reference

standard were also eligible for inclusion. Studies in which test strategies were compared head-to-head either in the same women, or between randomised groups were identified for inclusion in separate comparisons of test strategies. We excluded studies if they included less than five Down's syndrome cases, or more than 20% of participants were not followed up.

Data collection and analysis

We extracted data as test positive or test negative results for Down's and non-Down's pregnancies allowing estimation of detection rates (sensitivity) and false positive rates (1-specificity). We performed quality assessment according to QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria. We used hierarchical summary ROC meta-analytical methods or random-effects logistic regression methods to analyse test performance and compare test accuracy as appropriate. Analyses of studies allowing direct and indirect comparisons between tests were undertaken.

Main results

We included 56 studies (reported in 68 publications) involving 204,759 pregnancies (including 2113 with Down's syndrome). Studies were generally of good quality, although differential verification was common with invasive testing of only high-risk pregnancies. We evaluated 78 test combinations formed from combinations of 18 different tests, with or without maternal age; ADAM12 (a disintegrin and metalloprotease), AFP (alpha-fetoprotein), inhibin, PAPP-A (pregnancy-associated plasma protein A, ITA (invasive trophoblast antigen), free βhCG (beta human chorionic gonadotrophin), PlGF (placental growth factor), SP1 (Schwangerschafts protein 1), total hCG, progesterone, uE3 (unconjugated oestriol), GHBP (growth hormone binding protein), PGH (placental growth hormone), hyperglycosylated hCG, ProMBP (proform of eosinophil major basic protein), hPL (human placental lactogen), (free αhCG, and free ßhCG to AFP ratio. Direct comparisons between two or more tests were made in 27 studies.

Meta-analysis of the nine best performing or frequently evaluated test combinations showed that a test strategy involving maternal age and a double marker combination of PAPP-A and free ßhCG significantly outperformed the individual markers (with or without maternal age) detecting about seven out of every 10 Down's syndrome pregnancies at a 5% false positive rate (FPR). Limited evidence suggested that marker combinations involving PAPP-A may be more sensitive than those without PAPP-A.

Authors' conclusions

Tests involving two markers in combination with maternal age, specifically PAPP-A, free βhCG and maternal age are significantly better than those involving single markers with and without age. They detect seven out of 10 Down's affected pregnancies for a fixed 5% FPR. The addition of further markers (triple tests) has not been shown to be statistically superior; the studies included are small with limited power to detect a difference.

The screening blood tests themselves have no adverse effects for the woman, over and above the risks of a routine blood test. However some women who have a 'high risk' screening test result, and are given amniocentesis or chorionic villus sampling (CVS) have a risk of miscarrying a baby unaffected by Down's. Parents will need to weigh up this risk when deciding whether or not to have an amniocentesis or CVS following a 'high risk' screening test result.

PLAIN LANGUAGE SUMMARY

Screening tests for Down's syndrome in first three months of pregnancy

Background

Down's syndrome (also known as Down's or Trisomy 21) is an incurable genetic disorder that causes significant physical and mental health problems, and disabilities. However, there is wide variation in how Down's affects people. Some individuals are severely affected whilst others have mild problems and are able to lead relatively normal lives. There is no way of predicting how badly a baby might be affected.

Expectant parents are given the choice to be tested for Down's during pregnancy to assist them in making decisions. If a mother is carrying a baby with Down's, then there is the decision about whether to terminate or continue with the pregnancy. The information offers parents the opportunity to plan for life with a Down's child.

The most accurate tests for Down's involve testing fluid from around the baby (amniocentesis) or tissue from the placenta (chorionic villus sampling (CVS)) for the abnormal chromosomes associated with Down's. Both these tests involve inserting needles through the mother's abdomen and are known to increase the risk of miscarriage. Thus, the tests are not suitable for offering to all pregnant women. Rather, tests that measure markers in the mother's blood, urine or on ultrasound scans of the baby are used for screening. These screening tests are not perfect, they can miss cases of Down's and also give a 'high risk' test result to a number of women whose babies are not affected by Down's. Thus, pregnancies identified as 'high risk' using these screening tests require further testing using amniocentesis or CVS to confirm a diagnosis of Down's.

What we did

The aim of this review was to find out which of the blood screening tests done during the first three months of pregnancy are the most accurate at predicting the risk of a pregnancy being affected by Down's. We looked at 18 different blood markers that can be used alone or in combination, taken before 14 weeks gestation, thus creating 78 screening tests fro Down's. We found 56 studies, involving 204,759 pregnancies of which 2113 had pregnancies affected by Down's.

What we found

For the first 14 weeks of pregnancy, the evidence supports the use of the double test of two blood markers; pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotrophin (βhCG), in combination with the mother's age. This test detects around seven out of every 10 (68%) pregnancies affected by Down's. It is common practice to offer amniocentesis or CVS to women with a high risk test result. About one in 20 women (5%) having this test will have a 'high risk' result but most of these women will not be carrying a baby with Down's. We found for tests in the first 14 weeks of pregnancy, there is little evidence to support the use of serum tests made up of more than two blood markers.

Other important information to consider

The blood tests themselves have no adverse effects for the woman, over and above the risks of a routine blood test. However some women who have a 'high risk' screening test result, and are given amniocentesis or CVS have a risk of miscarrying a baby unaffected by Down's. Parents will need to weigh up this risk when deciding whether or not to have an amniocentesis or CVS following a 'high risk' screening test result.

BACKGROUND

This is one of a series of reviews on antenatal screening for Down's syndrome following a generic protocol (Alldred 2010) - see Published notes for more details.

Target condition being diagnosed

Down's syndrome

Down's syndrome affects approximately one in 800 live-born babies (Cuckle 1987a). It results from a person having three, rather than two, copies of chromosome 21, or the specific area of chromosome 21 implicated in causing Down's syndrome, as a result of trisomy or translocation. If not all cells are affected, the pattern is described as 'mosaic'. Down's syndrome can cause a wide range of physical and mental problems. It is the commonest cause of mental disability, and is also associated with a number of congenital malformations, notably affecting the heart. There is also an increased risk of cancers such as leukaemia, and numerous metabolic problems including diabetes and thyroid disease. Some of these problems may be life-threatening, or lead to considerable ill health, while some individuals with Down's syndrome have only mild problems and can lead a relatively normal life.

There is no cure for Down's syndrome, and antenatal diagnosis allows for preparation for the birth and subsequent care of a baby with Down's syndrome, or for the offer of a termination of pregnancy. Having a baby with Down's syndrome is likely to have a significant impact on family and social life, relationships and parents' work. Special provisions may need to be made for education and care of the child, as well as accommodating the possibility of periods of hospitalisation.

Definitive invasive tests (amniocentesis and chorionic villus sampling (CVS)) exist that allow the diagnosis of Down's syndrome before birth, but carry a risk of miscarriage. No test can predict the severity of problems a person with Down's syndrome will have. Noninvasive screening tests based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allow an estimate of the risk of a pregnancy being affected and provide parents with information to enable them to make choices about definitive testing. Such screening tests are used during the first and second trimester of pregnancy.

Screening tests for Down's syndrome

Initially, screening was determined solely by using maternal age to classify a pregnancy as high or low risk for trisomy 21, as it was known that older women had a higher chance of carrying a baby with Down's syndrome (Penrose 1933).

Further advances in screening were made in the early 1980s, when Merkatz et al. investigated the possibility that low maternal serum alpha-fetoprotein (AFP), obtained from maternal blood in the second trimester of pregnancy could be associated with chromosomal abnormalities in the fetus. Their retrospective case-control study showed a statistically significant relationship between fetal trisomy, such as Down's syndrome, and lowered maternal serum AFP (Merkatz 1984). This was further explored by Cuckle et al in a larger retrospective trial using data collected as part of a neural tube defect (NTD) screening project (Cuckle 1984). This work was followed by calculation of risk estimates using maternal serum AFP values and maternal age, which ultimately led to the introduction of the two screening parameters in combination (Alfirevic 2004).

In 1987, in a small case-control study of women carrying fetuses with known chromosomal abnormalities, Bogart and colleagues investigated maternal serum levels of human chorionic gonadotrophin (hCG) as a possible screening tool for chromosomal abnormalities in the second trimester (Bogart 1987). This followed the observations that low hCG levels were associated with miscarriages, which are commonly associated with fetal chromosomal abnormalities. They concluded that high hCG levels were associated with Down's syndrome and because hCG levels plateau at 18 to 24 weeks, that this would be the most appropriate time for screening. Later work suggested that the ß subunit of hCG was a more effective marker than total hCG (Macri 1990; Macri 1993).

Second trimester unconjugated oestriol (uE3), produced by the fetal adrenals and the placenta, was also evaluated as a potential screening marker. In another retrospective case-control study, uE3 was shown to be lower in Down's syndrome pregnancies compared with unaffected pregnancies. When used in combination with AFP and maternal age, it appeared to identify more pregnancies affected by Down's syndrome than AFP and age alone (Canick 1988).

Further work suggested that all three serum markers (AFP, hCG and uE3) showed even higher detection rates when combined with maternal age (Wald 1988a; Wald 1988b) and appeared to be a cost-effective screening strategy (Wald 1992a).

Two other serum markers, produced by the placenta, have been linked with Down's syndrome, namely pregnancy associated plasma protein A or PAPP-A, and first trimester Inhibin A. PAPP-A has been shown to be reduced in the first trimester of Down's syndrome pregnancies, with its most marked reduction in the early first trimester (Bersinger 1995). Inhibin A is high in the second trimester in pregnancies affected by Down's syndrome (Cuckle 1995; Wallace 1995). There are some issues concerning the biological stability and hence reliability of this marker, and the effect this will have on individual risk.

Screening and parental choice

Antenatal screening is used for several reasons (Alfirevic 2004), but the most important is to enable parental choice regarding pregnancy management and outcome. Before a woman and her partner opt to have a screening test, they need to be fully informed about the risks, benefits and possible consequences of such a test. This includes the choices they may have to face should the result show that the woman has a high risk of carrying a baby with Down's syndrome and implications of both false positive and false negative screening tests. They need to be informed of the risk of a miscarriage due to invasive diagnostic testing, and the possibility that a miscarried fetus may be chromosomally normal. If, following invasive diagnostic testing, the fetus is shown to have Down's syndrome, further decisions need to be made about continuation or termination of the pregnancy, the possibility of adoption and finally, preparation for parenthood. Equally, if a woman has a test that shows she is at a low risk of carrying a fetus with Down's syndrome, it does not necessarily mean that the baby will be born with a normal chromosomal make up. This possibility can only be excluded by an invasive diagnostic test (Alfirevic 2003).The decisions that may be faced by expectant parents inevitably engender a high level of anxiety at all stages of the screening process, and the outcomes of screening can be associated with considerable physical and psychological morbidity. No screening test can predict the severity of problems a person with Down's syndrome will have.

Index test(s)

This review examined serum screening tests used in the first trimester of pregnancy (up to 14 weeks' gestation) comprised of the following 18 individual markers; a disintegrin and metalloprotease 12 (ADAM12), AFP, inhibin, PAPP-A, invasive trophoblast antigen (ITA), free βhCG, placental growth factor (PlGF), Schwangerschafts protein 1 (SP1), total hCG, progesterone, uE3, growth hormone binding protein (GHBP), placental growth hormone (PGH), hyperglycosylated hCG, proform of eosinophil major basic protein (ProMBP), human placental lacto-

gen (hPL), free alpha human chorionic gonadotrophin (αhCG), and free ßhCG to AFP ratio. These markers can be used individually, in combination with age, and can also be used in combination with each other. The risks are calculated by comparing a woman's test result for each marker with values for an unaffected population, and multiplying this with her age-related risk. Where several markers are combined, risks are computed using risk equations (often implemented in commercial software) that take into account the correlational relationships between the different markers and marker distributions in affected and unaffected populations.

Alternative test(s)

Down's syndrome can be detected during pregnancy with invasive diagnostic tests such as amniocentesis or CVS, with or without prior screening. The ability to determine fetal chromosomal make up (also known as a karyotype) from amniotic fluid samples was demonstrated in 1966 by Steele and Breg (Steele 1966), and the first antenatal diagnosis of Down's syndrome was made in 1968 (Vaklenti 1968). Amniocentesis is an invasive procedure that involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks' gestation. CVS involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks' gestation. Amniocentesis and CVS are both methods of obtaining fetal chromosomal material, which are then used to diagnose Down's syndrome. Both tests use ultrasound scans to guide placement of the needle. Amniocentesis carries a risk of miscarriage in the order of 1%; transabdominal CVS may carry a similar risk (Alfirevic 2003).

Rationale

This is one of a suite of Cochrane reviews, the aim of which is to identify all screening tests for Down's syndrome used in clinical practice, or evaluated in the research setting, in order to try to identify the most accurate test(s) available, and to provide clinicians, policy-makers and women with robust and balanced evidence on which to base decisions about interpreting test results and implementing screening policies to triage the use of invasive diagnostic testing.

There are many different screening tests which are available and offered which will be the subject of additional Cochrane reviews (currently in preparation or published (Alldred 2012)), and there are other reviews looking at this area. Tests to be assessed in Cochrane reviews include second trimester serum tests; urine tests; first trimester ultrasound markers; tests that combine serum and ultrasound markers; and tests that combine markers from the first trimester with markers from the second trimester. Second trimester ultrasound markers have been assessed in a previous systematic review (Smith-Bindman 2001).

The topic has been split into several different reviews to allow for greater ease of reading and greater accessibility of data, and also to allow the reader to focus on separate groups of tests, for example, first trimester serum tests alone, first trimester serum and ultrasound, second trimester serum alone, first and second trimester serum combinations, with or without ultrasound markers; and urine markers alone. An overview review will compare the best tests, focusing on commonly used strategies and the best tests from each of these categories. This review is written with the global perspective in mind, rather than to conform with any specific local or national policy, as not all tests will be available in all areas where screening for Down's syndrome is carried out.

A systematic review of second trimester ultrasound markers in the detection of Down's syndrome fetuses was published in 2001 that concluded that nuchal fold thickening may be useful in detecting Down's syndrome, but that it was not sensitive enough to use as a screening test. The review concluded that the other second trimester ultrasound markers did not usefully distinguish between Down's syndrome and pregnancies without Down's syndrome (Smith-Bindman 2001). There has yet to be a systematic review and meta-analysis of the observed data on serum, urine and first trimester ultrasound markers, in order to draw rigorous and robust conclusions about the diagnostic accuracy of available Down's syndrome screening tests.

OBJECTIVES

The aim of this review was to estimate and compare the accuracy of first trimester serum markers for the detection of Down's syndrome in the antenatal period, both as individual markers and as combinations of markers. Accuracy is described by the proportion of fetuses with Down's syndrome detected by screening before birth (sensitivity or detection rate), and the proportion of women with a low risk (normal) screening test result who subsequently had a baby unaffected by Down's syndrome (specificity).

Investigation of sources of heterogeneity

We planned to investigate whether a uniform screening test is suitable for all women, or whether different screening methods are more applicable to different groups, defined by advanced maternal age, ethnic groups and aspects of the pregnancy and medical history such as multiple pregnancy, diabetes and family history of Down's syndrome. We also considered whether there existed evidence of overestimation of test accuracy in studies evaluating risk equations in the derivation sample rather than in a separate validation sample.

METHODS

Criteria for considering studies for this review

Types of studies

We included studies in which all women from a given population had one or more index test(s) compared to a reference standard. Both consecutive series and diagnostic case-control study designs were included. Randomised trials where individuals were randomised to different screening strategies and all verified using a reference standard were also eligible for inclusion. Studies in which test strategies were compared head-to-head, either in the same women, or between randomised groups were identified for inclusion in separate comparisons of test strategies. Studies were excluded if they included less than five Down's syndrome cases, or more than 20% of participants were not followed up.

Participants

Pregnant women at less than 14 weeks' gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome in their pregnancy were eligible. Studies were included if the pregnant women were unselected, or if they represented groups with increased risk of Down's syndrome, or difficulty with conventional screening tests including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

Index tests

The following 18 index tests were examined; ADAM12, AFP, inhibin, PAPP-A, ITA, free βhCG, PlGF, SP1, total hCG, progesterone, uE3, GHBP, PGH, hyperglycosylated hCG, ProMBP, hPL, free αhCG, and free ßhCG to AFP ratio, and combinations of these markers combined with maternal age.

We looked at comparisons of tests used in isolation and in 78 various combinations. These included single (one marker), double (two markers), triple (three markers), quadruple (four markers) and quintuple (five markers) tests, some of which were adjusted for maternal age.

Where tests were used in combination, we looked at the performance of test combinations according to predicted probabilities computed using risk equations and dichotomised into high risk and low risk.

Target conditions

Down's syndrome in the fetus due to trisomy, translocation or mosaicism.

Reference standards

We considered several reference standards, involving chromosomal verification and postnatal macroscopic inspection. Chromosomal verification is considered preferential but because of the risks involved, often not feasible. Where macroscopic inspection or examination raises a question about the possibility of an individual being affected by Down's syndrome, in clinical practice this is usually confirmed or refuted by formal karyotyping.

Amniocentesis and CVS are invasive chromosomal verification tests undertaken during pregnancy. They are highly accurate but the process carries a 1% miscarriage rate, and therefore they are only used in pregnancies considered to be high risk of Down's, or on the mother's request. All other types of testing (postnatal examination, postnatal karyotyping, birth registers and Down's syndrome registers) are based on information available at the end of pregnancy. For the purposes of meta-analysis they are considered equivalent. The greatest concern is not their accuracy, but the loss of the pregnancy to miscarriage between the timing of serum testing and the reference standard. Miscarriage with cytogenetic testing of the fetus is included in the reference standard where available.

We anticipated that older studies, and studies undertaken in older women were more likely to have used invasive chromosomal verification tests in all women. Studies undertaken in younger women and more recent studies were likely to use differential verification as they often only used prenatal karyotypic testing on fetuses considered screen positive or high risk according to the screening test; the reference standard for most unaffected infants is likely to be observation of a phenotypically normal baby. Although the accuracy of this combined reference standard is considered high, it is methodologically a weaker approach because pregnancies that miscarry between the index test and birth are likely to be lost from the analysis, and miscarriage is more likely to occur in Down's than normal pregnancies.

Search methods for identification of studies

We used one generic search strategy to identify studies for all reviews in this series

Electronic searches

We applied a sensitive search strategy to search the following databases using the text words and MeSH terms detailed in Appendix 1, adapting the search strategy for each different database.

Databases searched included:

- MEDLINE via OVID (1980 to 25 August 2011)
- Embase via Dialog Datastar (1980 to 25 August 2011)
- BIOSIS via EDINA (1985 to 25 August 2011)
- CINAHL via OVID (1982 to 25 August 2011)

- The Database of Abstracts of Reviews of Effectiveness (25 August 2011)
- MEDION (25 August 2011)
- The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (www.ifcc.org/) (25 August 2011)
- The National Research Register (Archived 2007)
- Health Services Research Projects in Progress database (HSRPROJ) (25 August 2011)

The search strategy combined three sets of search terms (see Appendix 1). The first set was made up of named tests, general terms used for screening/diagnostic tests and statistical terms. Note that the statistical terms were used to increase sensitivity and were not used as a methodological filter to increase specificity. The second set was made up of terms that encompass Down's syndrome and the third set made up of terms to limit the testing to pregnant women. All terms within each set were combined with the Boolean operator OR and then the three sets were combined using AND. The terms used were a combination of subject headings and free text terms. The search strategy was adapted to suit each database searched.

We attempted to identify cumulative papers which reported data from the same data set, and contacted authors to obtain clarification of the overlap between data presented in these papers, in order to prevent data from the same women being analysed more than once.

Searching other resources

In addition, we examined references cited in studies identified as being potentially relevant, and those cited by previous reviews. We contacted authors of studies where further information was required.

We carried out forward citation searching of relevant items, using the search strategy in ISI citation indices, Google scholar and Pubmed 'related articles'.

We did not apply language restrictions to the search.

Data collection and analysis

Selection of studies

Two review authors screened the titles and abstracts (where available) of all studies identified by the search strategy. We obtained full-text versions of studies identified as being potentially relevant and two review authors independently assessed these for inclusion, using a study eligibility screening pro forma according to the prespecified inclusion criteria. Any disagreement between the two review authors was settled by consensus, or where necessary, by a third party.

Data extraction and management

A data extraction form was developed and piloted using a subset of 20 identified studies. Two review authors independently extracted data, and where disagreement or uncertainty existed, a third review author validated the information extracted.

Data on each marker were extracted as binary test positive/test negative results for Down's and non-Down's pregnancies, with a high-risk result-as defined by each individual study-being regarded as test positive (suggestive or diagnostic of Down's syndrome), and a low-risk result being regarded as test negative (suggestive of absence of Down's syndrome). Where results were reported at several thresholds, we extracted data at each threshold.

We made a note of those in special groups that posed either increased risk of Down's syndrome or difficulty with conventional screening tests, including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

Assessment of methodological quality

We used a modified version of the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool (Whiting 2003), a quality assessment tool for use in systematic reviews of diagnostic accuracy studies, to assess the methodological quality of included studies. We anticipated that a key methodological issue would be the potential for bias arising from the differential use of invasive testing and follow-up for the reference standard according to index test results, bias arising due to higher loss to miscarriage if false negatives than true negatives. We chose to code this issue as originating from differential verification in the QUADAS tool: we are aware that it could also be coded under delay in obtaining the reference standard, and reporting of withdrawals. We omitted the QUADAS item assessing quality according to length of time between index and reference tests, as Down's syndrome is either present or absent rather than a condition that evolves and resolves, and disregarding the differential reference standard issue, thus any length of delay is acceptable. Two review authors assessed each included study separately. Any disagreement between the two authors was settled by consensus, or where necessary, by a third party. Each item in the QUADAS tool was be marked as 'yes', 'no' or 'unclear', and scores are presented graphically and in tables. We did not use a summary quality score. See Appendix 3 for QUADAS questionnaire.

Statistical analysis and data synthesis

We initially examined each test or test strategy at each of the common risk thresholds used to define test positivity by plotting estimates of sensitivity and specificity from each study on forest plots and in receiver operating characteristic (ROC) space. Test strategies were selected for further investigation if they were evaluated in four or more studies or, if there were two or three studies, but the individual study results indicated performance likely to be superior to a sensitivity of 70% and specificity of 90%.

Estimation of average sensitivity and specificity

The analysis for each test strategy was undertaken first restricting to studies that reported a common threshold to estimate average sensitivity and specificity for each test at each threshold. Although data on all thresholds were extracted, we present only key common thresholds from the literature, close to risks of 1:384, 1:250 and the 5% false positive rate (FPR), unless other thresholds were more commonly reported. Where combinations of tests were used in a risk score we extracted the result for the test combination using the risk score and not the individual components that made up the test.

We undertook meta-analyses using hierarchical summary ROC (HSROC) models, which included estimation of random-effects in accuracy and threshold parameters when there were four or more studies. Where there were fewer than four studies and the studies reported test performance at a common threshold, we computed average sensitivity and specificity values by using univariate fixed-effect or random-effects logistic regression models to average logit sensitivity and logit specificity separately because of insufficient number of studies to reliably estimate all the parameters in the HSROC model. It is common in this field for studies to report sensitivity for a fixed specificity (usually a 5% FPR). This removes the requirement to account for the correlation between sensitivity and specificity across studies by using a bivariate meta-analytical method since all specificities are the same value. Thus, at a fixed specificity value, logit sensitivities were pooled using a univariate random-effects logistic regression model. This model was further simplified to a fixed-effect model when there were only two or three studies and heterogeneity was not observed on the SROC plot. All analyses were undertaken using the NLMIXED procedure in SAS (version 9.2; SAS Institute, Cary, NC) and the xtmelogit command in Stata version 11.2 (Stata-Corp, College Station, TX, USA).

Comparisons between tests

We made comparisons between tests, first by utilising all available studies, selecting one threshold from each study to estimate a summary ROC curve without restricting to a common threshold, and second, by making pair-wise comparisons using studies that compared tests in the same mothers (direct head-to-head comparison). The threshold was chosen for each study according to the following order of preference a) the risk threshold closest to 1 in 250; b) a multiples of the median (MoM) or presence/absence threshold; c) the performance closest to a 5% FPR or 95th percentile. The 5% FPR was chosen as a cut-off point as this is the cut-off most commonly reported in the literature.

For the analysis that included data from all studies, we compared test strategies in a single HSROC model, including two indicator terms for each test to allow for differences in accuracy and threshold. There was no indication of differing SROC curve shape between tests and so a single SROC shape parameter was included in the model, such that the fitted SROC curves did not cross. The initial meta-analyses of individual test strategies indicated there were differences in the variability of the accuracy parameter such that the assumption of equal variances may not be justifiable. We attempted to fit a model with separate variance terms for each test strategy for the accuracy parameter but the model did not converge. We therefore restricted the meta-analysis that compared the accuracy of the different test strategies to only studies that used a 5% FPR threshold so that we could fit a univariate random effects logistic regression model that allowed for a separate variance term for the random-effects of logit sensitivity for each test. Using non-linear combinations of the parameter estimates from this model, we derived ratios of sensitivities for each pair of tests included in the model and obtained their corresponding 95% confidence interval (CI) by using the delta method. We used likelihood ratio tests to assess the statistical significance of differences in sensitivity between tests.

For direct comparisons between each pair of tests at the 5% FPR threshold, we used a separate model for each pair-wise comparison and pooled logit sensitivities using a univariate random-effects model. As studies rarely reported data cross-classified by both tests for Down's and normal pregnancies, the analytical method did not take full account of the pairing of test results, but the restriction to direct head-to-head comparisons should have removed the potential confounding of test comparisons with other features of the studies. The strength of evidence for differences in performance of test strategies relied on evidence from both the direct and indirect comparisons.

Investigations of heterogeneity

We planned to undertake investigations of heterogeneity if there were 10 or more studies available for a test. We planned to investigate the effect of a covariate by adding covariate terms to the HSROC model to assess differences in accuracy and threshold.

Sensitivity analyses

Mothers with pregnancies identified as high risk for Down's syndrome by serum testing are often offered immediate definitive testing by amniocentesis, whereas those considered low risk are assessed for Down's syndrome by inspection at birth. Such delayed and differential verification will introduce bias most likely through there being greater loss to miscarriage in the Down's syndrome pregnancies that were not detected by the serum testing (the false negative diagnoses). Testing and detection of miscarriages is impractical in many situations, and no clear data are available on the magnitude of these miscarriage rates.

To account for the possible bias introduced by such a mechanism, we planned to perform sensitivity analyses by increasing the percentage of false negatives in studies where delayed verification in test negatives occurred (Mol 1999). We planned to incrementally

increase the percentage from 10% to 50%, the final value representing a scenario where a third or more Down's pregnancies than normal pregnancies were likely to miscarry, thought to be higher than the likely value. We intended to conduct the sensitivity analyses on the analysis investigating the effect of maternal age on test sensitivity.

RESULTS

Results of the search

The search for the whole suite of reviews identified a total of 15,394 papers, once the results from each bibliographic database were combined and duplicates were removed. After screening out obviously inappropriate papers based on their title and abstract, 1145 papers remained and we obtained full-text copies for formal assessment of eligibility. From these, a total of 269 papers were deemed eligible and were included in the suite of reviews. We included a total of 56 studies (reported in 68 publications) in this review of first trimester serum screening, involving 204,759 pregnancies, of which 2113 were Down's syndrome pregnancies.

A total of 78 different test strategies or combinations, at one or more thresholds, were evaluated in the 56 studies. These tests were produced from combinations of 18 different serum tests with and without maternal age; ADAM12, AFP, inhibin, PAPP-A, ITA, free βhCG, PlGF, SP1, total hCG, progesterone, uE3, GHBP, PGH, hyperglycosylated hCG, ProMBP, hPL, free αhCG, and free ßhCG to AFP ratio. Strategies evaluated included three quintuple tests, three quadruple tests, 12 triple tests, 27 double tests and 15 single tests in combination with maternal age, and three triple tests, five double tests and 10 single tests without maternal age.

The following combinations evaluated included four or more studies.

Double tests with maternal age

1. Free ßhCG, AFP and maternal age (five studies; 5160 women including 174 Down's syndrome pregnancies)
2. Free ßhCG, PAPP-A and maternal age (31 studies; 158,878 women including 1430 Down's syndrome pregnancies)

Single tests with maternal age

1. Free ßhCG and maternal age (nine studies; 16,656 women including 549 Down's syndrome pregnancies)
2. PAPP-A and maternal age(six studies; 13,742 women including 409 Down's syndrome pregnancies)

Single tests without maternal age

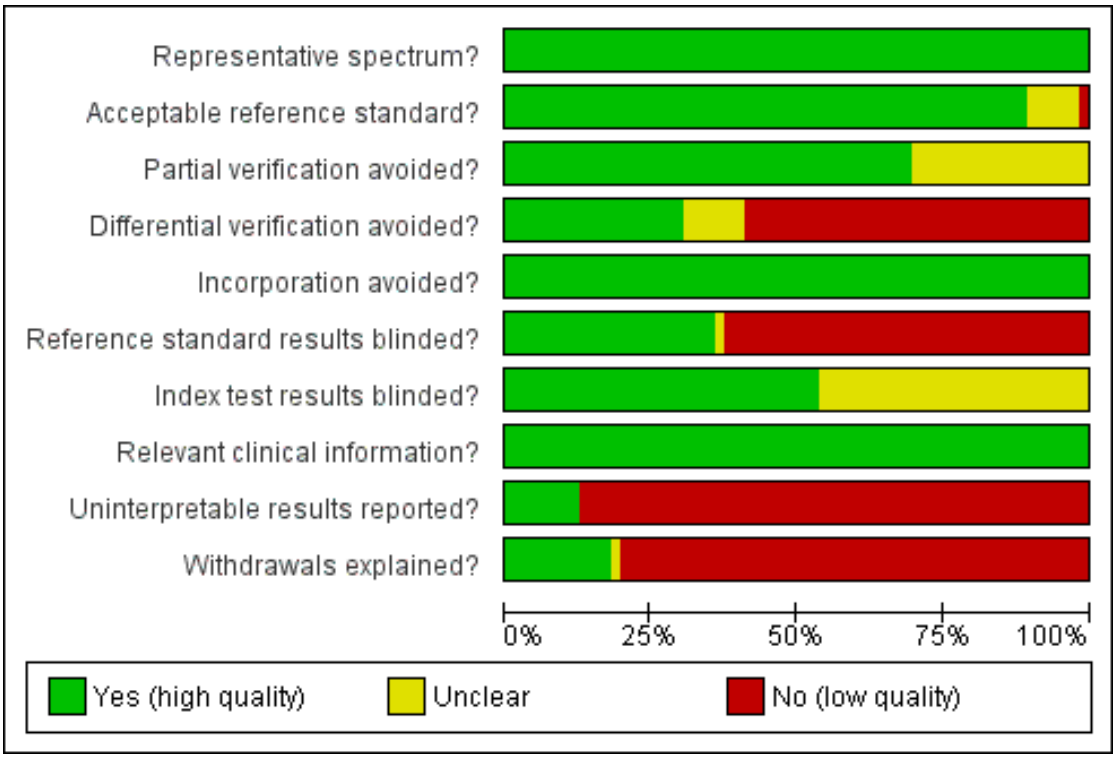
1. Free ßhCG (four studies; 4280 women including 390 Down's syndrome pregnancies)
2. PAPP-A (six studies; 25,510 women including 430 Down's syndrome pregnancies)

Of the remaining test combinations, seven were evaluated in three studies, 17 were evaluated in two studies and the remainder were evaluated in single studies only.

Methodological quality of included studies

We judged the methodological quality of the studies to be high in most categories (Figure 1). Due to the nature of testing for Down's syndrome screening and the potential side effects of invasive testing, differential verification is almost universal in the general screening population, as most women whose screening test result is defined as low risk will have their screening test verified at birth, rather than by invasive diagnosis in the antenatal period. Additionally, it was not always possible to ascertain from the included studies whether or not the results of index tests and reference standards were blinded. It would be difficult to blind clinicians performing invasive diagnostic tests (reference standards) to the index test result, unless all women received the same reference standard, which would not be appropriate in most scenarios. Any biases secondary to a lack of clinician blinding are likely to be minimal.

Figure 1. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.



Where details of completeness of follow-up were poorly reported, most studies seemed to assume 100% follow-up. However, there will inevitably be losses to follow-up due to women moving out of area, for example. Studies sometimes accounted for these and it is unlikely that there were enough losses to follow-up to have introduced significant bias. There was likely under-ascertainment of miscarriage, and very few papers accounted for miscarriage, or performed tissue karyotyping in pregnancies resulting in miscarriage. Some studies attempted to adjust for predicted miscarriage rate and the incidence of Down's syndrome in this specific population, but most did not. We have not attempted to adjust for expected miscarriage rate in this review. There is a higher natural miscarriage rate in the first trimester, however this will be uniform across studies and therefore unlikely to introduce significant bias. Some studies that provided estimates of risk using multivariable equations used the same data set to evaluate performance of the risk equation as was used to derive the equation. This is often thought to lead to over-estimation of test performance.

Findings

The findings of the 21 most common and/or best performing test strategies are given in Summary of findings 1. The remaining 57 strategies are briefly summarised in Summary of findings 2. The test strategies evaluated by four or more studies are detailed below.

1) Free ßhCG, PAPP-A and maternal age (double test)

Results for this double test were derived from 31 studies (Biagiotti 1998; Brambati 1994; Christiansen 2005; Christiansen 2007a; Christiansen 2009; Christiansen 2010; Cowans 2010; Crossley 2002a; De Graaf 1999a; Forest 1997; Gyselaers 2005; Haddow 1998; Kagan 2009; Kozlowski 2007 GC; Kozlowski 2007 PC; Krantz 2000; Muller 2003a; Niemimaa 2001a; O'Leary 2006; Orlandi 1997; Sahota 2010; Schaelike 2009; Scott 2004; Spencer 1999a; Torring 2010; Tsukerman 1999; Valinen 2007; Wald 2003a; Wapner 2003; Wojdemann 2005; Zaragoza 2009), and included 158,878 women in whom 1430 pregnancies were known to be affected by Down's syndrome. Seven studies contributed over 10,000 pregnancies each to the data (Crossley 2002a; Gyselaers 2005; Kagan 2009; Krantz 2000; O'Leary 2006; Sahota 2010; Schaelike 2009). Studies presented data for cut-points of 5% FPR (Biagiotti 1998; Brambati 1994; Cowans 2010; De Graaf 1999a; Forest 1997; Haddow 1998; Kagan 2009; Sahota 2010; Spencer 1999a;Sahota 2010; Torring 2010; Tsukerman 1999; Wald

2003a; Wapner 2003; Zaragoza 2009), 1:250 risk (Christiansen 2005; Christiansen 2007a; Christiansen 2009; Christiansen 2010; Crossley 2002a; Kagan 2009; Muller 2003a; Niemimaa 2001a; Torring 2010; Valinen 2007; Wojdemann 2005), and 1:300 risk (Kozlowski 2007 GC; Kozlowski 2007 PC; Schaelike 2009). At a cut-point of 5% FPR (17 studies), the sensitivity wa s estimated as 68% (95% confidence interval (CI) 65 to 71) and the specificity at 95% (95% CI 95 to 95). At a cut-point of 1:250 FPR (11 studies), the sensitivity was estimated as 73% (95% CI 67 to 79) and the specificity as 93% (95% CI 91 to 94).

2) Free ßhCG, AFP and maternal age (double test)

Results for this double test were derived from five studies (Benattar 1999; Biagiotti 1995; Forest 1995; Tsukerman 1999; Wald 2003a), and included 5160 women in whom 174 pregnancies were known to be affected by Down's syndrome. Two contributed over 1000 pregnancies each to the data (Benattar 1999; Tsukerman 1999. Studies presented data for cut-points of 5% FPR (Biagiotti 1995; Tsukerman 1999; Wald 2003a), 1:250 risk (Benattar 1999) and 1:384 risk (Forest 1995). At a cut-point of 5% FPR (three studies), the sensitivity was estimated as 49% (95% CI 39 to 60) and the specificity as 95% (95% CI 94 to 96).

3) PAPP-A and maternal age (single test)

Results for this single test were derived from six studies (Biagiotti 1998; Brambati 1993; Forest 1997; Krantz 2000; Spencer 1999a; Wald 2003a), and included 13,742 women in whom 409 pregnancies were known to be affected by Down's syndrome. Krantz 2000 was the largest study, contributing over 10,000 pregnancies to the data. Studies presented data for cut-points of 5% FPR (Biagiotti 1998; Brambati 1993; Forest 1997; Spencer 1999a; Wald 2003a) and 1:105 risk (Krantz 2000). At a cut-point of 5% FPR (five studies), the sensitivity was estimated as 55% (95% CI 46 to 63) and the specificity as 95% (95% CI 94 to 96).

4) Free ßhCG and maternal age (single test)

Results for this single test were derived from nine studies (Biagiotti 1995; Biagiotti 1998; Brambati 1994; Forest 1995; Forest 1997; Krantz 2000; Noble 1995; Spencer 1999a; Wald 2003a), and included 16,656 women in whom 549 pregnancies were known to be affected by Down's syndrome. Krantz 2000 contributed over 10,000 pregnancies to the data. Studies presented data for cut-points of 5% FPR (Biagiotti 1995; Biagiotti 1998; Brambati 1994; Forest 1997, Noble 1995; Spencer 1999a; Wald 2003a), 1:384 risk (Forest 1995) and 1:105 risk (Krantz 2000). At a cut-point of 5% FPR (seven studies), the sensitivity was estimated as 42% (95% CI 36 to 48) and the specificity as 95% (95% CI 94 to 96).

5) PAPP-A alone (single test without maternal age)

Results for this single test were derived from six studies (Brambati 1993; Brameld 2008; Brizot 1994; Casals 1996; Spencer 1999a; Wald 2003a), and included 25,510 women in whom 430 pregnancies were known to be affected by Down's syndrome. Brameld 2008 was the largest study contributing over 20,000 pregnancies to the data. Studies presented data for cut-points of 5% FPR (Brambati 1993; Brizot 1994; Casals 1996; Spencer 1999a; Wald 2003a) and $\leq$ 5th percentile (Brameld 2008). At a cut-point of 5% FPR (four studies), the sensitivity was estimated as 52% (95% CI 39 to 65) and the specificity as 95% (95% CI 94 to 96).

6) Free ßhCG alone (single test without maternal age)

Results for this single test were derived from four studies (Casals 1996; Noble 1997; Spencer 1999a; Wald 2003a), and included 4280 women in whom 390 pregnancies were known to be affected by Down's syndrome. Studies were all of a similar size. Studies presented data at a 5% FPR. At this cut-point, the sensitivity was estimated as 25% (95% CI 18 to 34) and the specificity as 95% (95% CI 94 to 96).

7) Other test combinations

Of the 73 test combinations evaluated in three or fewer studies, several test combinations demonstrated estimated sensitivities of more than 70% and estimated specificities of more than 90%. Twelve of these were evaluated in single studies (Summary of findings 2), however, three test combinations were evaluated in two or more studies.

1. A triple test of**PAPP-A, free ßhCG, AFP and maternal age** was evaluated in three studies (Muller 2003a; Tsukerman 1999; Wald 2003a), had an estimated sensitivity of 74% (95% CI 65 to 81) at a cut-point of 5% FPR.
2. A triple test of **ADAM 12, PAPP-A, free ßhCG and maternal age** was evaluated in three studies (Christiansen 2010; Torring 2010; Valinen 2009), had an estimated sensitivity of 74% (95% CI 63 to 83) at a cut-point of 5% FPR.
3. A triple test of **PlGF, PAPP-A, free ßhCG and maternal age** was evaluated in two studies (Cowans 2010; Zaragoza 2009), had an estimated sensitivity of 76% (95% CI 69 to 82) at a cut-point of 5% FPR.

Comparative analysis of the nine selected test strategies

We chose to estimate detection rates at a 5% FPR, in common with much of the literature. Figure 2 shows point estimates of detection rates for a 5% FPR based on all available data for all nine test combinations described above, and the confidence intervals at a fixed 5% FPR. For example, the plot shows that for the double test with a marker combination of free βhCG, AFP and maternal age, the

estimated detection rate at a 5% FPR was 49% (95% CI 39 to 60) based on data from three studies with 157 affected cases and 2992 total participants. The test combinations in Figure 2 are ordered according to decreasing detection rates. The single test strategies with and without maternal age (PAPP-A alone; free βhCG alone, PAPP-A and maternal age, and free βhCG and maternal age) have the worst performance, whereas, the triple test strategies (ADAM 12, PAPP-A, free βhCG and maternal age; PAPP-A, free βhCG, AFP and maternal age) have the highest performance. In between lie the double tests (free βhCG, PAPP-A and maternal age; free βhCG, AFP and maternal age). However, it should be noted that the confidence intervals on these estimates are wide and overlap for the lower performing five strategies, suggesting that any of the differences observed may be explicable by chance.

Figure 2. Detection rates (sensitivity) at a 5% false positive rate for the nine selected test strategies. Each circle represents the summary sensitivity for a test strategy and the size of each circle is proportional to the number of Down's cases. The estimates are shown with 95% confidence intervals. The test strategies are ordered on the plot according to decreasing detection rate. The number of studies, cases and women included for each test strategy are shown on the horizontal axis.A = Age, PlGF, PAPP-A and free ßhCG; B = Age, PAPP-A, free ßhCG and AFP; C = Age, ADAM 12, PAPP-A and free ßhCG; D = Age, PAPP-A and free ßhCG ; E = Age, PAPP-A; F = PAPP-A; G = Age, free ßhCG and AFP ; H = Age, free ßhCG; I = Free ßhCG

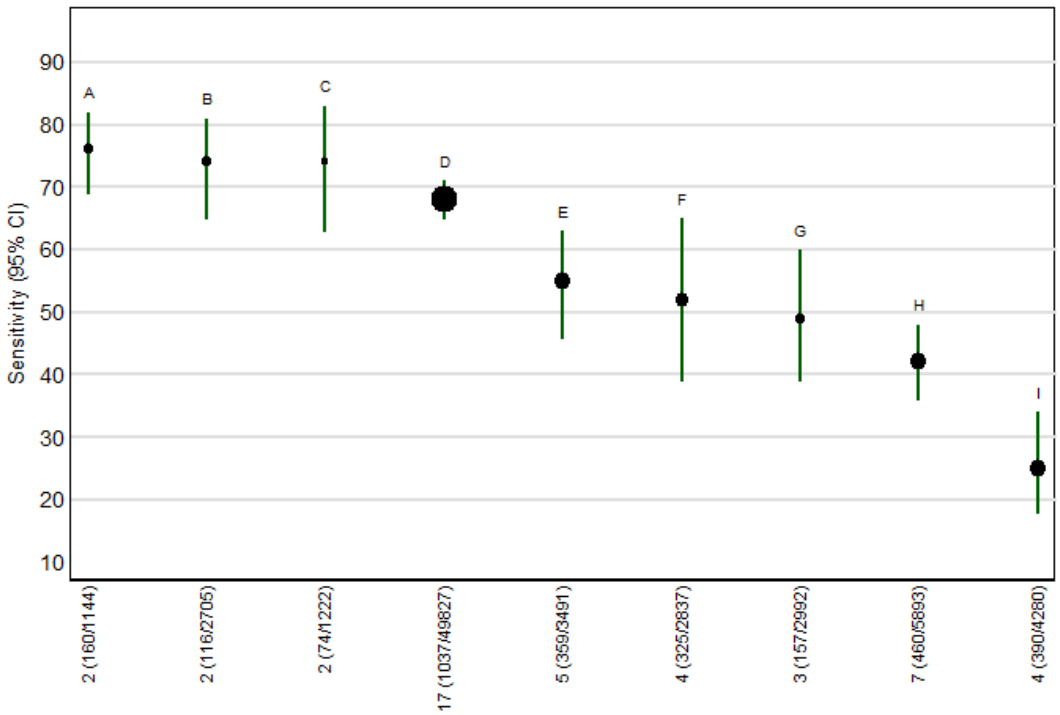


Table 1 shows pair-wise direct comparisons (head-to-head) where studies were available. Such comparisons are regarded as providing the strongest evidence as they compare tests within pregnancies and are thus unconfounded. The table shows the ratios of sensitivities with 95% CIs and P values (P < 0.05 being considered a statistically significant difference) for each test comparison, the number of studies (*K*) for which data were available. The table shows that the sensitivity of the single test combinations (PAPP-A alone, free βhCG alone, PAPP-A and maternal age, and free βhCG and maternal age) tended to be significantly worse (P < 0.05) than the double and triple tests where data are available. The double test comprised of PAPP-A, free βhCG and maternal age appears to have significantly better (P = 0.004) test accuracy than the double test comprised of free βhCG, AFP and maternal age. Otherwise, there was no strong evidence of significant improvements in sensitivity with the addition of a third marker. However, most comparisons in this table are based on only single studies and are unlikely to be powered to detect differences in detection rates.

Table 2 shows the same comparisons made using all available data (as used to create Figure 2). Results are in agreement with the direct comparisons, and in addition, showed that the triple test comprised of PlGF, PAPP-A, free ßhCG and maternal age is significantly better (P = 0.024) than the double test comprised of PAPP-A, free βhCG and maternal age. However, these comparisons are potentially confounded by differences between the studies, and are based on small numbers of studies.

Investigation of heterogeneity and sensitivity analyses

The key characteristics of the 56 included studies is summarised in Table 3 with further details available in the Characteristics of included studies table. Only one test combination- PAPP-A, free ßhCG and maternal age (17 studies) was evaluated by 10 or more studies but there were no data for investigation of the effect of maternal age or any other potential source of heterogeneity. The planned sensitivity analyses were also not possible.

Summary of findings

Review Question	What is the accuracy of serum-based markers for Down's syndrome screening in the first trimester?
Population	Pregnant women at less than 14 weeks' gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome. Most studies were undertaken in women identified to be high risk based on maternal age
Settings	All settings
Numbers of studies, pregnancies and Down's syndrome cases	56 studies (68 publications) involving 204,759 pregnancies of which 2113 were Down's syndrome pregnancies
Index tests	18 serum markers (ADAM12, AFP, inhibin, PAPP-A, ITA, free βhCG, PlGF, SP1, total hCG, progesterone, uE3, GHBP, PGH, hyperglycosylated hCG, ProMBP, hPL, free αhCG, and free ß hCG to AFP ratio) singly or in combination with or without maternal age
Reference standards	Chromosomal verification (amniocentesis and CVS undertaken during pregnancy, and postnatal karyotyping) and postnatal macroscopic inspection
Study limitations	35 studies used selective chromosomal verification during pregnancy, and were at risk of under-ascertainment of Down's syndrome cases due loss of the pregnancy to miscarriage between the serum test and the reference standard

Tests with at least 70% sensitivity and at least 95% specificity

Test strategy	Studies	Women (cases)	Sensitivity (95% CI)	Specificity (95% CI)	Test*
Test strategies (with or without maternal age) evaluated by a single study					
Without maternal age					
Double tests					
PAPP-A and AFP	1	96 (16)	81 (54 to 96)	95 (88 to 99)	
PAPP-A and ITA	1	344 (24)	71 (49 to 87)	95 (92 to 97)	
Triple tests					

PAPP-A, free ß hCG and ITA	1	344 (24)	75 (53 to 90)	95 (92 to 97)
PlGF, PAPP-A and free ß hCG	1	699 (90)	72 (62 to 81)	95 (93 to 97)
With maternal age				
Double tests				
Free ß hCG and SP1	1	60 (14)	71 (42 to 92)	96 (85 to 99)
PAPP-A and Hyperglycosylated hCG	1	10775 (23)	74 (52 to 90)	95 (95 to 95)
Triple tests				
PAPP-A, free ß hCG and Inhibin	1	1110 (85)	74 (63 to 83)	95 (94 to 96)
PAPP-A, SP1 and ProMBP	1	192 (15)	73 (45 to 92)	95 (91 to 98)
hPL, PAPP-A and free ß hCG (1:250 risk)	1	183 (47)	77 (62 to 88)	95 (90 to 98)
Quadruple tests				
GHBP, PGH, PAPP-A and free ß hCG (1:250 risk)	1	335 (74)	76 (64 to 85)	95 (91 to 97)
Quintuple tests				
PAPP-A, free ß hCG, AFP, uE3 and Inhibin	1	1110 (85)	78 (67 to 86)	95 (94 to 96)
PAPP-A, total hCG, AFP, uE3 and Inhibin	1	1110 (85)	73 (62 to 82)	95 (94 to 96)
Test strategies (with or without maternal age) evaluated by at least two studies				

Free ßhCG	4	4280 (390)	25 (18 to 34)	95 (94 to 96)	P <0.001
PAPP-A	4	2837 (325)	52 (39 to 65)	95 (94 to 96)	
Age, free ßhCG	7	5893 (460)	42 (36 to 48)	95 (94 to 96)	
Age, PAPP-A	5	3491 (359)	55 (46 to 63)	95 (94 to 96)	
Age, free ßhCG and AFP	3	2992 (157)	49 (39 to 60)	95 (94 to 96)	
Age, PAPP-A and free ßhCG	17	49827 (1037)	68 (65 to 71)	95 (95 to 95)	
Age, PAPP-A, free ßhCG and AFP	2	2705 (116)	74 (65 to 81)	95 (94 to 96)	
Age, ADAM 12, PAPP-A and free ßhCG	2	1222 (74)	74 (63 to 83)	95 (94 to 96)	
Age, PIGF, PAPP-A and free ßhCG	2	1144 (160)	76 (69 to 82)	95 (93 to 96)	

*Likelihood ratio test for the difference in sensitivity between the nine test strategies that were formally compared in a single meta-analytic model.

ADAM12: a disintegrin and metalloprotease; **AFP:** alpha-fetoprotein; αhCG: alpha human chorionic gonadotrophin; **ßhCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **CVS:** chorionic villus sampling; **GHBP:** growth hormone binding protein; **hCG:** human chorionic gonadotrophin; **hPL:** human placental lactogen; **ITA:** invasive trophoblast antigen; **PAPP-A:** pregnancy-associated plasma protein A; **PGH:** placental growth hormone; **PIGF:** placental growth factor; **PROMBP:** proform of eosinophil major basic protein; **SPI:** Schwangerschafts protein 1; **uE3:** unconjugated oestriol

Test strategy	Studies	Women (cases)	Sensitivity (95% CI)	Specificity (95% CI)	Threshold
Without maternal age					
Single tests					
AFP	2	2248 (104)	10 (4 to 21)	95	5% FPR
ADAM 12	1	579 (17)	41 (18 to 67)	95 (93 to 97)	5% FPR
Free ß hCG to AFP ratio	1	476 (9)	11 (0 to 48)	98 (96 to 99)	0.25 MoM
Inhibin	3	2098 (184)	19 (4 to 58)	95	5% FPR
PlGF	1	699 (90)	28 (19 to 38)	95 (93 to 97)	5% FPR
Total hCG	3	2098 (184)	19 (4 to 58)	95	5% FPR
SP1	3	1080 (53)	32 (1 to 96)	95	5% FPR
uE3	1	1110 (85)	13 (7 to 22)	95 (94 to 96)	5% FPR
Double tests					
Free ß hCG and AFP	1	1138 (19)	16 (3 to 40)	95 (94 to 96)	5% FPR
Free ß hCG and Inhibin	1	876 (76)	30 (20 to 42)	95 (93 to 96)	5% FPR
PAPP-A and free ß hCG	2	795 (106)	64 (50 to 76)	95	5% FPR
Triple tests					
Total hCG, free αhCG and progesterone	1	129 (17)	53 (28 to 77)	96 (90 to 99)	0.34 MoM
With maternal age					

Single tests					
ADAM 12	2	703 (46)	67 (46 to 83)	91 (87 to 94)	1:400 risk
AFP	2	1397 (126)	33 (23 to 46)	95	5% FPR
Free αhCG	1	512 (12)	25 (5 to 57)	89 (86 to 91)	1:384 risk
GHBP	1	335 (74)	27 (17 to 39)	95 (91 to 97)	1:250 risk
hPL	1	183 (47)	45 (30 to 60)	93 (88 to 97)	1:250 risk
Inhibin	1	1110 (85)	32 (22 to 43)	95 (94 to 96)	5% FPR
ITA	1	278 (54)	48 (34 to 62)	95 (91 to 98)	5% FPR
PGH	1	335 (74)	41 (29 to 53)	94 (91 to 97)	1:250 risk
PlGF	1	699 (90)	43 (33 to 54)	95 (93 to 97)	5% FPR
ProMBP	1	181 (25)	36 (18 to 57)	94 (89 to 97)	1:250 risk
SP1	2	804 (29)	38 (22 to 56)	95	5% FPR
Total hCG	1	512 (12)	33 (10 to 65)	94 (92 to 96)	1:384 risk
uE3	1	512 (12)	33 (10 to 65)	86 (83 to 89)	1:384 risk
Double tests					
ADAM 12 and PAPP-A	1	691 (46)	61 (45 to 75)	95 (93 to 97)	5% FPR
AFP and free αhCG	1	512 (12)	33 (10 to 65)	87 (83 to 89)	1:384 risk
AFP and total hCG	1	512 (12)	33 (10 to 65)	93 (90 to 95)	1:384 risk

AFP and uE3	1	512 (12)	42 (15 to 72)	87 (84 to 90)	1:384 risk
Free ß hCG and free αhCG	1	512 (12)	42 (15 to 72)	94 (91 to 96)	1:384 risk
Free ß hCG and Inhibin	1	1110 (85)	44 (33 to 55)	95 (94 to 96)	5% FPR
Free ß hCG and total hCG	1	512 (12)	25 (5 to 57)	93 (90 to 95)	1:384 risk
Free ß hCG and uE3	1	287 (41)	61 (45 to 76)	95 (92 to 97)	5% FPR
GHBP and free ß hCG	1	335 (74)	61 (49 to 72)	92 (88 to 95)	1:250 risk
GHBP and PAPP-A	1	335 (74)	66 (54 to 77)	93 (89 to 96)	1:250 risk
GHBP and PGH	1	335 (74)	47 (36 to 59)	93 (90 to 96)	1:250 risk
hPL and free ß hCG	1	183 (47)	68 (53 to 81)	94 (89 to 97)	1:250 risk
hPL and PAPP-A	1	183 (47)	55 (40 to 70)	94 (89 to 97)	1:250 risk
PAPP-A and AFP	2	2705 (116)	63 (50 to 74)	95	5% FPR
PAPP-A and Inhibin	1	1110 (85)	68 (57 to 78)	95 (94 to 96)	5% FPR
PAPP-A and ITA	2	622 (78)	62 (46 to 75)	95	5% FPR
PGH and free ß hCG	1	335 (74)	64 (52 to 74)	93 (89 to 96)	1:250 risk
PGH and PAPP-A	1	335 (74)	65 (53 to 76)	93 (89 to 96)	1:250 risk
Total hCG and free αhCG	1	512 (12)	42 (15 to 72)	92 (89 to 94)	1:384 risk
Total hCG and Inhibin	1	1110 (85)	34 (24 to 45)	95 (94 to 96)	5% FPR
Total hCG and PAPP-A	2	4327 (133)	66 (54 to 76)	95	5% FPR

Total hCG and uE3	1	512 (12)	42 (15 to 72)	92 (89 to 94)	1:384 risk
uE3 and free αhCG	1	512 (12)	33 (10 to 65)	89 (86 to 91)	1:384 risk
Triple tests					
AFP, free αhCG and uE3	1	512 (12)	58 (28 to 85)	82 (79 to 85)	1:384 risk
Free ß hCG, AFP and uE3	1	287 (41)	66 (49 to 80)	95 (92 to 97)	5% FPR
GHBP, PAPP-A and free ß hCG	1	335 (74)	76 (64 to 85)	94 (91 to 97)	1:250 risk
PAPP-A, total hCG and Inhibin	1	1110 (85)	69 (58 to 79)	95 (94 to 96)	5% FPR
PGH, PAPP-A and free ß hCG	1	335 (74)	76 (64 to 85)	94 (91 to 97)	1:250 risk
Total hCG, AFP and uE3	1	512 (12)	42 (15 to 72)	91 (88 to 94)	1:384 risk
Quadruple tests					
Free ß hCG, total hCG, AFP and uE3	1	512 (12)	50 (21 to 79)	92 (89 to 94)	1:384 risk
Total hCG, AFP, uE3 and free αhCG	1	512 (12)	50 (21 to 79)	90 (87 to 92)	1:384 risk
Quintuple tests					
Free ß hCG, total hCG, AFP, uE3 and free αhCG	1	512 (12)	33 (10 to 65)	90 (87 to 92)	1:384 risk

AFP: alpha-fetoprotein; αhCG: alpha human chorionic gonadotrophin; **ß hCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **FPR:** false positive rate; **GHBP:** growth hormone binding protein; **hCG:** human chorionic gonadotrophin; **hPL:** human placental lactogen; **ITA:** invasive trophoblast antigen; **PAPP-A:** pregnancy-associated plasma protein A; **PGH:** placental growth hormone; **PIGF:** placental growth factor; **PROMBP:** proform of eosinophil major basic protein; **SPI:** Schwangerschafts protein 1; **uE3:** unconjugated oestriol

DISCUSSION

Summary of main results

The systematic review found a large number of studies evaluating first trimester Down's syndrome serum screening tests, including studies evaluating the commonly used double test. Few studies were available to evaluate the performance of test strategies involving newer markers, such as ADAM 12, and few studies provided unconfounded comparisons of test strategies by applying and comparing several strategies using the same serum sample, the majority of studies only evaluating a single test combination. A summary of results for the nine most common and best performing strategies is given in Summary of findings 1, briefer details for the remaining strategies are given in Summary of findings 2.
Three key findings were noted.

1. The double test comprised of PAPP-A, free βhCG and maternal age appears to have significantly better ($P < 0.05$) test accuracy than the double test comprised of free βhCG, AFP and maternal age, and the single tests (both the markers alone and in combination with maternal age). This test detects around seven out of every 10 Down's affected pregnancies for a fixed 5% FPR. By comparison, the double test comprised of free βhCG, AFP and maternal age, and single tests alone and in combination with maternal age detects between two and five out of every 10 Down's affected pregnancies for a fixed 5% FPR.
2. Whilst the triple test combinations show the highest detection rates, they were not shown to be statistically superior to the double test comprised of PAPP-A, free βhCG and maternal age. Whilst some significant differences between these categories of tests were noted in the indirect comparisons, the potential for confounding is of concern. Estimates suggest that triple test combinations may detect between seven and eight out of every 10 Down's syndrome pregnancies at a 5% FPR, however these estimates are based on data from two or three studies evaluating small numbers of women. It is difficult to make strong recommendations on the use of triple tests, as we cannot rule out possible differences due to the limited power there is to detect a difference.
3. The evidence for higher numbers of markers shows similar detection rates to double and triple markers, but are based on data from one study only, therefore further evaluation of these tests is required. Furthermore, there are other combinations of double markers that show similar detection rates to standard double markers commonly used in clinical practice, which may warrant further study.

Strengths and weaknesses of the review

This review is the first comprehensive review of first trimester serum screening. We examined papers from around the world, covering a wide cross-section of women in varying populations. We contacted authors to verify data where necessary to give as complete a picture as possible, while trying to avoid replication of data.
There were a number of factors that made meta-analysis of the data difficult, which we tried to adapt for, in order to allow for comparability of data presented in different studies.

1. There were many different cut-points used to define pregnancies as high or low risk for Down's syndrome. This means that direct comparison is more difficult than if all studies used the same cut-point to dichotomise their populations. This is less of an issue for first trimester serum screening, compared to second trimester serum screening, as the majority of authors chose a cut-point of 5% FPR.
2. There were many different risk equations and software applications in use for combination of multiple markers, which were often not described in the papers. This means that risks may be calculated by different formulae and they may not be directly comparable for this reason. It is possible that this is responsible for unexplained heterogeneity in results.
3. Different laboratories and clinics run different assays and use different machines and methods. This may influence raw results and subsequent risk calculations. Many laboratories have a quality assessment or audit trail, however, this may not necessarily be standard across the board. For example, how many assays are run, how often medians are calculated and adjusted for a given population and how quickly samples are tested from initially being taken.
4. Few papers made direct comparisons between tests, making it difficult to detect if a real difference exists between tests (i.e. how different tests perform in the same population). There were differences in populations, with assay medians being affected, for example, by race. It is not certain whether it is appropriate to make comparisons between populations which are inherently different.
5. We were unable to perform the investigations of heterogeneity that we had originally intended to because the data simply were not available. The vast majority of papers looking at pregnancies conceived by IVF, affected by diabetes, multiple gestation or a family history of Down's syndrome involved unaffected pregnancies only.

In addition, the search for this review was last updated in August 2011, and it is possible that new studies may have been published which have not been included. Since the search was completed we have kept a watching brief on outputs and are not aware of any studies with substantial sample sizes which could substantially affect the findings.

Applicability of findings to the review question

Potentially, when planning screening policy or a clinical screening programme, clinicians and policy makers need to make decisions

about a finite number of tests or type of tests that can be offered. These policies are often driven by both the needs of a specific population and by financial resources. Economic analysis was considered to be outside of the scope of this review. Many of the tests examined as part of this review are already commercially available and in use in the clinical setting. The studies were carried out on populations of typical pregnant women and therefore, the results should be considered comparable with most pregnant populations encountered in every day clinical practice.

We were unable to extract information about harms of testing, information about miscarriage rates and uptake of definitive testing as the data were not available the majority of the time. Whilst it is unlikely that major differences between the tests evaluated here exist in terms of direct harms of testing, as they are all based on a single blood sample, differences in accuracy may lead to differences in the use of definitive testing and its consequent adverse outcomes.

In some countries with a defined screening policy (i.e. the UK), first trimester screening plays a major role, although usually in combination with first trimester ultrasound scanning. In others however, there may only be a limited range of tests or markers available, often second trimester markers, rather than first trimester markers. The results of this review should be interpreted and applied in the context of test availability and local restrictions, populations or policies.

AUTHORS' CONCLUSIONS

Implications for practice

The evidence supports the use of the first trimester double test comprised of PAPP-A, free βhCG and maternal age, there is little evidence to recommend the use of first trimester serum tests with three or more markers, however the data available on these tests are limited, and based on generally small populations of women. We would not recommend that these tests be introduced into wider clinical practice without careful consideration of cost.

The review has shown that tests involving two or three markers in combination with maternal age are significantly better than those involving one marker. We would therefore recommend that one marker tests are not used for Down's syndrome screening. The choice of multiple markers will depend on the availability of certain assays in local laboratories. On the basis of this review we would recommend the combination of PAPP-A, free βhCG and maternal age, as it significantly outperforms free βhCG, AFP and maternal age, and is widely available. The data for other test combinations limits our ability to make any other recommendations about specific test combinations. Alternative screening methods should also be considered (i.e. use of ultrasound markers in the first trimester) when making policy decisions, and are the subject of other reviews in this suite.

The screening blood tests themselves have no adverse effects for the woman, over and above the risks of a routine blood test. However some women who have a 'high risk' screening test result, and are given amniocentesis or CVS have a risk of miscarrying a baby unaffected by Down's. Parents will need to weigh up this risk when deciding whether or not to have an amniocentesis or CVS following a 'high risk' screening test result.

Implications for research

Further evaluations of test combinations involving three or more markers are required to determine whether their apparent advantages are not chance findings.

Future studies should ensure that adequate sample sizes are recruited, and take opportunities to make comparisons of test performance testing several alternative test combinations on the same serum samples. Such direct comparison removes issues of confounding when making test comparisons, and allows a clear focus on testing the incremental benefit of increasingly complex and expensive testing strategies. The reporting of studies of test accuracy can be improved and more closely adhere to the standards for the reporting of diagnostic accuracy studies (STARD) guideline. Three key aspects of this are 1) formally testing the statistical significance of differences in test performance in direct comparisons and estimating incremental changes in detection rates (together with confidence intervals), 2) clearly reporting the number of mothers studied and their results, and 3) reporting the numbers of women who are lost to follow-up. Many authors reported results of extrapolating findings to age-standardised national cohorts to demonstrate the performance of the test, and failed to report the actual numbers studied and evaluated.

For the purposes of meta-analysis and to allow for comparisons to be made between different tests and combinations, we would recommend the publication of consensus standard algorithms for estimating risk, and reporting of test performance at a standard set of thresholds. This would be difficult to achieve and implement, but an attempt at consensus should be made.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Baviera 2010

Clinical features and settings	Routine screening.
Participants	579 participants: 17 cases and 562 controls matched for gestational age Italy - single centre. December 2006-May 2009. Pregnant women. Mean maternal age 35.3 years (cases) and 30.4 years (controls) Singleton pregnancies. 7-10 and 14-17 weeks' gestation.
Study design	Case- control study.
Target condition and reference standard(s)	Down's syndrome: 17 cases (14 identified by amniocentesis, 3 from follow-up to birth) Reference standards: amniocentesis or follow-up to birth.
Index and comparator tests	Frozen serum samples tested for: First trimester and second trimester ADAM12s (time resolved fluorescence immunoassay, DELFIA assay kit, Perkin Elmer Life and Analytical Sciences) First trimester PAPP-A (details not reported). Second trimester AFP, uE3 and hCG (details not reported).
Follow-up	Details of follow-up not reported.
Aim of study	To demonstrate the potential value of repeated measures of ADAM12s for the screening of Down's syndrome
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.

Baviera 2010 (*Continued*)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Benattar 1999

Clinical features and settings	Routine screening.
Participants	1656 participants. France - single centre. January to December 1995. Singleton pregnancies. Pregnant women. Mean age 32 years (16-46 years). Enrolled before 13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 5 cases. Reference standards: amniocentesis due to maternal age > 38 years (6.1% or women) . Karyotyping encouraged for women with positive result on 1 or more index test. No details of reference standard for index test negative women
Index and comparator tests	Maternal age. NT at 12-14 weeks (Toshiba SSA 270), cut-point 1/250. First trimester (12-14 weeks) serum AFP and free ßhCG (Elsa AFP and Elsa free ßhCG; Cis-Bio International) Second trimester (15-18 weeks) serum AFP and total hCG (AFP-2T and hCG-60; Ortho-Clinical Diagnostics)
Follow-up	Details of follow-up not stated. Unclear whether women were followed up to birth
Aim of study	To evaluate the sequential combination of ultrasound screening for fetal aneuploidy at 11-14 weeks with maternal biochemistry at 12-14 and 15-18 weeks of gestation

Benattar 1999 (*Continued*)

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	Yes	12 patients were lost to follow-up due to miscarriages.

Biagiotti 1995

Clinical features and settings	High-risk referral for invasive testing.
Participants	287 participants: 41 cases and 246 controls matched for maternal and gestational age, and duration of sample storage (from cohort of 4452 participants undergoing invasive testing) Italy - single centre. Dates not specified. Pregnant women. Singleton pregnancies. 8-12 weeks' gestation.

Biagiotti 1995 (*Continued*)

Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 41 cases. Reference standards: amniocentesis or CVS.
Index and comparator tests	Maternal age. Frozen samples tested for: First trimester AFP - AFP-M-K S Kit. First trimester uE3 - Amersham Amerlex M. First trimester Intact hCG - Hybritech tandem. First trimester free ßhCG - ELSA Free beta hCG CIS.
Follow-up	100% karyotyping.
Aim of study	Evaluate first trimester maternal serum AFP, uE3 and hCG to assess the efficacy of different combinations of these markers on a screening test for Down's syndrome in the first trimester
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had invasive testing.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (lab analysis blinded)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice

Biagiotti 1995 (*Continued*)

Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Biagiotti 1998

Clinical features and settings	High-risk referral for invasive testing.
Participants	232 participants: 32 cases and 200 randomly selected controls (selected from series of 3731 women) Italy - single centre. July 1993 to December 1996. Pregnant women. Singleton pregnancies. 10 to 13 weeks' gestation.
Study design	Retrospective case-control study.
Target condition and reference standard(s)	Down's syndrome: 32 cases. Reference standards: amniocentesis or CVS.
Index and comparator tests	Maternal age. First trimester NT (in longitudinal section of the fetus with caliper measurements to the nearest 0.1 mm) First trimester serum PAPP-A (Amerlex-M PAPP-A IRMA, Ortho-Clinical Diagnostics) First trimester serum free ßhCG (Elsa9free B-hCG CIS).
Follow-up	100% karyotyping.
Aim of study	To evaluate the potential effectiveness of maternal serum PAPP-A and free beta hCG in combination with NT measurement in the first trimester of pregnancy
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.

Biagiotti 1998 (*Continued*)

Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Unclear	No details of withdrawals given.

Brambati 1993

Clinical features and settings	HIgh-risk referral for invasive testing.
Participants	522 participants. Italy. Dates not specified. Pregnant women. Median age 38 years (20-47 years). Singleton pregnancies. 6-11 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 14 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen blood sample tested for: FIrst trimester serum PAPP-A (radio-immunoassay).
Follow-up	100% karyotyping. 47 women who miscarried prior to CVS were excluded from the study
Aim of study	To assess the relationship between maternal serum PAPP-A in the first trimester and the outcome of pregnancy by karyotype

Brambati 1993 (*Continued*)

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Brambati 1994

Clinical features and settings	HIgh-risk referral for invasive testing.
Participants	102 participants: 13 case and 89 randomly selected controls matched for gestational age Italy. Dates not specified. Pregnant women. 8-12 weeks' gestation.
Study design	Case-control study.

Brambati 1994 (*Continued*)

Target condition and reference standard(s)	Down's syndrome: 13 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum samples tested for: FIrst trimester PAPP-A (radioimmunoassay, as described in Sinosich 1982) First trimester free ßhCG (radioimmunoassay, CIS, UK).
Follow-up	100% karyotyping.
Aim of study	To report the results for the combined measurement of serum PAPP-A and free-ßhCG in women attending for prenatal diagnosis
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical testing conducted blind to karyotype results)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements

Withdrawals explained? All tests	No	No details of withdrawals given.

Brameld 2008

Clinical features and settings	Routine screening.
Participants	22,280 participants with complete screening results and outcome data August 2001-October 2003. Australia - State-wide screening programme evaluation. Pregnant women. Median maternal age 31 years (range 14-47 years), 20% ≥ 35 years Singleton pregnancies. 10-14 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 60 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester PAPP-A, free ßhCG and NT (details not reported) Risk cut-point 1:300.
Follow-up	Data on outcome from the Western Australia Midwives data collection, Birth Defects Registry and hospital morbidity and mortality data
Aim of study	To identify first trimester indicators of adverse pregnancy outcomes
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.

Brameld 2008 (*Continued*)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Brizot 1994

Clinical features and settings	High-risk referral for invasive testing.
Participants	393 participants: 45 cases of Down's syndrome and 348 controls matched for crown rump length, maternal age and storage time UK. Dates not specified. Pregnant women. Median age 38 years (22-45 years). Singleton pregnancies. 10-13 weeks' gestation.
Study design	Retrospective case-control study.
Target condition and reference standard(s)	Down's syndrome: 45 cases. Reference standard: fetal karyotyping.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First Trimester PAPP-A (Double sandwich time resolved immunofluorometric assay with chelated europium as a label. Antibody to PAPPA binding Ig was polyclonal rabbit IgG in stabilised solution)
Follow-up	100% karyotyping
Aim of study	To determine if the risk for fetal trisomies during the first trimester of pregnancy can be derived by combining data from maternal serum PAPP-A and fetal NT thickness
Notes	Cases were pre-selected for increased NT thickness.

Brizot 1994 (*Continued*)

Table of Methodological Quality		
Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical testing conducted blind to karyotype results)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Casals 1996

Clinical features and settings	High-risk referral for invasive testing.
Participants	1138 participants. Spain. 1990-1993. Pregnant women. 94.4% of women aged > 35 years. Singleton pregnancies. 10-13 weeks' gestation.
Study design	Retrospective case-control study.

Casals 1996 (*Continued*)

Target condition and reference standard(s)	Down's syndrome: 19 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester serum free ßhCG (IMx microparticle enzyme immunoassay technology) (19 case and > 80 control samples) First trimester serum AFP (Stratus fluorometric enzyme immunoassay) (19 case and > 80 control samples) First trimester serum PAPP-A (radioimmunoassay) (only for 16 case and 80 control samples)
Follow-up	100% karyotyping.
Aim of study	To examine the value of ßhCG, AFP and PAPP-A in biochemical screening for Down's syndrome in 19 women carrying Down's syndrome versus normal pregnancies
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index test interpreted without knowledge of reference standard results (PAPP-A testing conducted blind to CVS results but presence of blinding is not stated for free ßhCG and AFP)

Casals 1996 (*Continued*)

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Christiansen 1999

Clinical features and settings	High-risk referral for invasive testing.
Participants	181 participants (for first trimester serum samples): 25 cases and 156 controls matched for length of storage Denmark. Dates not specified. Pregnant women. 5-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 25 cases. Reference standard: karyotyping.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First and second trimester ProMBP (2 site immunoradiometric assay samples reduced and alkylated and added to microtitre wells coated with monoclonal antibody J13 6B6)
Follow-up	100% karyotyping.
Aim of study	To examine whether the maternal serum concentration of ProMBP was influenced by the presence of a Down's syndrome fetus. To evaluate its potential as a screening marker for Down's syndrome in the first and second trimester of pregnancy. To examine the performance characteristics of a serum screening programme using ProMBP in combination with age as risk markers
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.

Christiansen 1999 (*Continued*)

Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Christiansen 2004

Clinical features and settings	Routine screening.
Participants	334 participants: 156 cases, 546 control samples (348 control women, 198 of these were sampled from the same women in first and second trimesters) Denmark. Dates not specified. Pregnant women. Singleton pregnancies. 4-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 156 cases. Reference standard: CVS (for 120 of cases of Down's) or follow-up to birth (for 36 of cases of Down's)
Index and comparator tests	Maternal age. Frozen serum samples tested for: First/second trimester hCG and AFP (AutoDELFIA analytical system)

	First/second trimester PAPP-A and SP1 (in-house sandwich immunoassays) First/second trimester ProMBP (2 site immunoradiometric assay (IRMA)) First/second trimester free ßhCG and some AFP (dual label kit)
Follow-up	The Danish Cytogenetic Central Registry was routinely used to ascertain that none of the controls were pregnancies with a chromosomally diseased fetus
Aim of study	To evaluate 6 markers of fetal Down's syndrome pregnancies (includes first trimester markers)
Notes	Unclear criteria for the selection of controls.

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Christiansen 2005

Clinical features and settings	Screening programmes for syphilis and Down's syndrome.
Participants	108 participants: 27 cases of Down's syndrome and 81 controls Denmark - Statens Serum Institute. Dates not specified. 5-11 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 27 cases (18 diagnosed in 2nd trimester, 9 at birth) Reference standard: karyotyping.
Index and comparator tests	Maternal age. First trimester (week 11-14) NT. Frozen samples tested for: First trimester (week 5-11) 1T Inhibin A (dimer assay kit MCA 950KZZ, Serotec) First trimester (week 5-11) ßhCG (available for some samples) First trimester (week 5-11) PAPP-A (available for some samples) (combined PAPP-A and B-hCG TrIFMA assay) Risk cut-points of 1:100, 1:250 and 1:400. Performance assessed with SPlus algorithm.
Follow-up	All diagnosis were verified by karyotyping.
Aim of study	To investigate whether first trimester Inhibin A can be used in the first trimester for Down's syndrome screening
Notes	Identified through the Danish central cytogenetic registry as part of quality assurance programme

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women had a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

Christiansen 2005 (*Continued*)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of NT results.
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Christiansen 2007a

Clinical features and settings	Routine screening.
Participants	183 participants: 47 cases and 136 controls matched for gestational age Dates not reported. Denmark - Statens Serum Institute. Pregnant women. Singleton pregnancies. Median age cases 37.7 years (24-48 years) and controls 36.4 years (22-44 years) 8-14 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 47 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. Fresh serum samples tested for: First trimester PAPP-A and free βhCG (AutoDelfia, PerkinElmer, Turku or Kryptor, Brahms) Frozen serum samples tested for: First trimester human placental lactogen (hPL) (hPL ELISA, enzyme immunoassay (EIA) -1283, DRG Instruments GmBH)
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry
Aim of study	To examine the potential of human placental lactogen as a first trimester maternal serum screening marker for fetal Down's syndrome
Notes	

Christiansen 2007a (*Continued*)

Table of Methodological Quality		
Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Christiansen 2009

Clinical features and settings	Routine screening.
Participants	335 participants: 74 cases and 261 controls matched for length of sample storage and maternal age Denmark - screening programme. Dates not reported. Pregnant women. Singleton pregnancies. Median maternal age cases 37.5 years and controls 36.4 years 8-13 weeks' gestation.
Study design	Case-control study.

Christiansen 2009 (*Continued*)

Target condition and reference standard(s)	Down's syndrome: 74 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (details not reported). Fresh serum samples tested for: First trimester PAPP-A and free ßhCG (AutoDelfia, PerkinElmer, Turku or Kryptor, Brahms) Frozen serum samples tested for: First trimester placental growth hormone (PGH) (double monoclonal ELISA, DSL-10-19 200, Diagnostic Systems Laboratory Inc) First trimester growth hormone binding protein (GHBP) (Enzyme-amplified ELISA, DSL-10-48 100, Diagnostic Systems Laboratory Inc)
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry
Aim of study	To examine the potential of placental growth hormone and growth hormone binding protein as maternal serum screening markers for Down's syndrome
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice

Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Christiansen 2010

Clinical features and settings	Routine screening.
Participants	531 participants: 28 cases and 503 controls. Denmark - screening programme. Dates not specified. Pregnant women. Singleton pregnancies. Median age cases 36 years (range 25-44 years) and controls 29 years (range 17-45 years) 8-14 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 28 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (details not reported). First trimester PAPP-A and free ßhCG (details not reported). First trimester ADAM12s (AutoDELFIA/Delfia ADAM12 Research kit 4025-0010, PerkinElmer Life and Analytical Sciences, on the 1235 AutoDELFIA automatic immunoassay system)
Follow-up	Cross-referencing with the Danish Cytogenetic Central Registry
Aim of study	To examine the efficiency of a second generation assay for ADAM12
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.

Christiansen 2010 (*Continued*)

Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Cowans 2010

Clinical features and settings	Routine screening.
Participants	445 participants: 70 cases and 375 controls matched for storage time and gestational age January 2007-October 2008. UK. Pregnant women. Singleton pregnancies. Mean maternal age cases 37.0 years (IQR 32.9-40.5 years) and controls 32.4 years (IQR 29.0-35.9 years) 11-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 70 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF certified sonographers). Fresh serum samples tested for: First trimester PAPP-A and free ßhCG (Kryptor analyser, Brahms) Frozen serum samples tested for: First trimester placental growth factor (PlGF) (Solid-phase, two-site fluoroimmunometric research assay (4083-0010) on 6000 DELFIA Xpress random access platform, PerkinElmer) Modelled on UK 2002 population data.

Follow-up	Karyotype and results for pregnancy outcome were received from cytogenetics laboratories and maternity units where deliveries took place
Aim of study	To examine placental growth factor levels in first trimester maternal serum in trisomy 21 pregnancies and to investigate the potential value of PlGF in a first trimester screening test
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Crandall 1993

Clinical features and settings	High-risk referral for invasive testing.
Participants	893 participants. USA - 3 centres. Dates not specified. Pregnant women, 90% > 35 years. 11-15 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 11 cases. Reference standard: amniocentesis.
Index and comparator tests	Maternal age. Frozen samples tested for: First trimester serum AFP (Tandem E kit). First trimester serum uE3 (Amerlex M). First trimester serum hCG (Hybritech tandem E kit).
Follow-up	100% karyotyping.
Aim of study	To investigate whether hCG is a useful predictor of Down's syndrome between 11 and 15 weeks' gestation
Notes	Unclear criteria for the selection of controls.

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Amniocentesis.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results

Crandall 1993 (*Continued*)

Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	33 samples excluded because out of the date range or insufficient sample volume or information
Withdrawals explained? All tests	No	No details of withdrawals given.

Crossley 2002a

Clinical features and settings	Routine screening.
Participants	17,229 participants. UK - 15 centres. Dates not specified. Pregnant women with median age 29.9 years, 15.4% ≥ 35 years. 10-14 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 45 cases. Reference standards: CVS offered where women had high NT measurements, amniocentesis or follow-up to birth
Index and comparator tests	Maternal age. NT (FMF method) in 73% of patients. Clotted blood samples tested for: Free ßhCG and PAPP-A (Kryptor analyser) in 98.4% of patients
Follow-up	Reported that the outcome of all pregnancies was followed up
Aim of study	To evaluate the use of ultrasound measurements of fetal NT obtained in a routine antenatal clinic setting in combination with appropriate biochemical markers as a first trimester screening test for Down's syndrome
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description

Crossley 2002a (*Continued*)

Representative spectrum? All tests	Yes	Routine screening of typical pregnant population .
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	Report average success rate of NT (72.9%).
Withdrawals explained? All tests	Yes	Numbers of patients not undergoing NT and biochemical testing given

De Graaf 1999a

Clinical features and settings	High-risk referral for invasive testing.
Participants	292 participants (207 participants before 14 weeks' gestation): 37 cases and 255 controls matched for maternal age (within 2 years), gestational age (within 2 weeks) and duration of sample storage (within 2 months) The Netherlands - single centre. 1994-1997. Pregnant women. 9-15 weeks' gestation (in a few cases, blood samples for serum testing taken at 15-19 weeks)
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 37 cases (30 affected pregnancies in women with serum testing enrolled before 14 weeks' gestation) Reference standards: CVS or amniocentesis.

De Graaf 1999a (*Continued*)

Index and comparator tests	Maternal age. First trimester NT (FMF methods) with cut off > 3 mm. Frozen serum samples tested for: First trimester free ßhCG and AFP (DELFIA dual labelled time resolved fluorescent assay) First trimester serum PAPP-A (DELFIA research assay (CR61-105)) First trimester serum AFP.
Follow-up	100% karyotyping.
Aim of study	To determine the expected detection rate and false positive rate for Down's syndrome achievable by early pregnancy screening with combined measurements of serum PAPP-A, free beta hCG and fetal NT, with the addition of AFP
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women had a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	Yes	Failed to measure NT in 11 control women.
Withdrawals explained? All tests	No	No details of withdrawals given.

Forest 1995

Clinical features and settings	Routine screening.
Participants	1023 participants (512 first trimester participants). Canada - 6 centres. June 1989-October 1993. Pregnant women. 23 cases of Down's syndrome (12 in of women recruited first trimester and 11 in second trimester) 1000 control samples (100 for each gestational week from 9-18) matched to the age of the original cohort in which Down's cases were detected (n = 14,612) Mean maternal age 29.1 (SD 4.7) years, 10.7% aged ≥ 35 years Singleton pregnancies. 9-13 (first trimester) and 14-18 (second trimester) weeks' gestation
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 12 cases (first trimester). Reference standards: follow-up to birth.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester AFP and total hCG (Enzymum-Test enzyme immunoassay analyser, ES-300 analyser) First trimester uE3 (ultra sensitive radioimmunometric assay) First trimester free αhCG and free ßhCG (radioimmunometric assays) 3 different models used for risk calculation (Wald, Spencer and Ryall)
Follow-up	Review of maternal and neonatal charts in each centre.
Aim of study	Evaluate the impact of risk estimation parameters for screening for Down's syndrome during the first and second trimesters of pregnancy
Notes	3 different models used for risk calculation (Wald, Spencer and Ryall)

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if women received different reference standards.

Forest 1995 (*Continued*)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Forest 1997

Clinical features and settings	Routine screening.
Participants	518 participants. Canada - 6 centres. June 1989-January 1995. Pregnant women. 18 cases of Down's syndrome. 500 control samples (representative of the cohort from which they were taken: n = 10, 160) 100 for each gestational week from 9-13 weeks. Mean maternal age 27.9 years, 10.7% aged ≥ 35 years. Singleton pregnancies. 9-13 weeks' gestation at enrolment.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 18 cases. Reference standards: follow-up to birth.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester PAPP-A (fluorescence-linked immunosorbent assay) First trimester free ßhCG (radioimmunometric assay, Bioclone Australia) Risk cut-point 1:384.
Follow-up	Review of maternal and neonatal charts in each centre and consulting the database of the local cytogenetic laboratories

Forest 1997 (*Continued*)

Aim of study	To confirm the usefulness of free ßhCG and AFP as first trimester screening markers in a prospective study
Notes	Exclusion of cases of babies that died before 20 weeks' gestation. Unclear criteria (apart from age) for the selection of controls

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if women received different reference standards.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Gyselaers 2005

Clinical features and settings	Routine screening.
Participants	13,267 participants (13,207 participant received both NT test and serum testing) Belgium - multicentre study (35 centres). First Jan 2004-First April 2004 (data added to previous database from before 2003) Pregnant women. Singleton pregnancies.

Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 26 cases. Reference standards: amniocentesis, CVS and postnatal karyotyping
Index and comparator tests	Maternal age. First trimester serum PAPP-A (ELISA 2397, DRG International Inc) First trimester serum free ßhCG (free ßhCG IRMA K1P1001, BioSource Europe SA) Second trimester PAPP-A and free ßhCG. First trimester NT. Risk cut-points of 1:200 and 1:300.
Follow-up	Follow-up to birth reported by mail by obstetricians. Non-responding obstetricians contacted personally to obtain missing data. Cases of miscarriages (n = 49) and other fetal chromosomal abnormalities excluded from the study. Unclear if other patients were lost to follow-up
Aim of study	To evaluate the performance of a first trimester fetal aneuploidy screening programme
Notes	Women with miscarriages or cases of other chromosomal defects were excluded from the study. 9 live births of babies with Down's syndrome

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	Yes	Numbers of women excluded due to miscarriage or other chromosomal defects and numbers not undergoing NT and biochemical testing reported

Haddow 1998

Clinical features and settings	High-risk referral for invasive testing.
Participants	3217 participants. USA - 16 prenatal diagnostic centres. June 1994 to November 1996. Pregnant women aged 15-51 years (median 37 years). Singleton pregnancies. 9-14 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 48 cases. Reference standards: amniocentesis or CVS.
Index and comparator tests	Maternal age. Fresh serum sample tested for: First trimester serum hCG (hCG MAIA clone assay). First trimester serum PAPP-A (enzyme-linked immunosorbent assay, Dako) First trimester free ßhCG and AFP - Fluoroimmunoassay (DELFIA hAFP/Free beta hCG dual kit) First trimester uE3 (Ultrasensitive uE3 kit).
Follow-up	100% karyotyping.
Aim of study	To further examine the efficacy of serum and ultrasound screening for Down's syndrome in the first trimester and the possible advantages and disadvantages of screening at this time rather than in the second trimester
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.

Haddow 1998 (*Continued*)

Acceptable reference standard? All tests	Yes	Karyotyping.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women had the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Kagan 2009

Clinical features and settings	Routine screening.
Participants	56,954 participants with available outcome data. UK - multicentre study. July 1999 - April 2007. Pregnant women. Singleton pregnancies. Mean maternal age 35.4 years (14.1-52.2 years). 11-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 395 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT. First trimester fetal heart rate (pulsed-wave Doppler). First trimester nasal bone (FMF certified sonographers).

	First trimester ductus venous flow (FMF certified sonographers) First trimester flow across tricuspid valve (FMF certified sonographers) First trimester PAPP-A and free ßhCG (Kryptor, Brahms AG or Delfia Express, Perkin Elmer) Multiple publications with different test evaluations.
Follow-up	Karyotype results and details of pregnancy outcome were added to databases as they became available. Women without complete screening and outcome data (n = 3053, 5. 1%) were excluded from the study
Aim of study	To examine the performance of first-trimester screening for trisomies 21, 18 and 13 by maternal age, fetal NT thickness, fetal heart rate and maternal serum free ß-hCG and PAPP-A Other objectives in related publications.
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements

Kagan 2009 (*Continued*)

Withdrawals explained? All tests	No	No details of withdrawals given.

Kornman 1998

Clinical features and settings	High-risk referral for invasive testing.
Participants	The Netherlands - antenatal diagnosis unit. October 1990-February 1994. Pregnant women. 15 cases of Down's syndrome. 97 control samples (matched on gestational age, sample storage time and maternal age) Singleton pregnancies. 8-12 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 15 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester serum SP1 (modified commercial radioimmunoassay, RIA-gnost SP1))
Follow-up	100% karyotyping.
Aim of study	To compare SP1 levels in Down's syndrome versus normal pregnancies
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

Kornman 1998 (*Continued*)

Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Kozlowski 2007 GC

Clinical features and settings	Routine referral.
Participants	6906 participants with complete outcome data. Germany - gynaecologists practices. January 2000-December 2003. Pregnant women. Median maternal age 32 years (15-48 years), 26.4% $\geq$ 35 years 11-14 weeks' gestation.
Study design	Cohort study.
Target condition and reference standard(s)	Down's syndrome: 19 cases. Reference standard: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF certified gynaecologists). First trimester free ßhCG and PAPP-A (Kryptor analyser, Brahms) Risk cut-point 1:300.
Follow-up	Data on pregnancy outcome were obtained by contacting the patient or their general gynaecologist. Women without complete outcome data (36%) were excluded from the study
Aim of study	To evaluate and compare the screening performance for fetal trisomy 21 in the first trimester of pregnancy in general gynaecologists practices and specialised centres for prenatal care in Germany
Notes	

Table of Methodological Quality

Kozlowski 2007 GC (*Continued*)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	146 women (including 11 with Down's syndrome) excluded as results could not be assigned to gynaecologists or prenatal centre group.

Kozlowski 2007 PC

Clinical features and settings	Routine referral.
Participants	3862 participants with complete outcome data. Germany - tertiary level prenatal centres. January 2000-December 2003. Pregnant women. Median maternal age 34 years (14-46 years), 43.2% $\geq$ 35 years 11-14 weeks' gestation.
Study design	Cohort study.
Target condition and reference standard(s)	Down's syndrome: 26 cases. Reference standard: karyotyping or follow-up to birth.

Index and comparator tests	Maternal age. First trimester NT (FMF certified sonographers). First trimester free ßhCG and PAPP-A (Kryptor analyser, Brahms) Risk cut-point 1:300.
Follow-up	Data on pregnancy outcome were obtained by contacting the patient or their general gynaecologist. Women without complete outcome data (8%) were excluded from the study
Aim of study	To evaluate and compare the screening performance for fetal trisomy 21 in the first trimester of pregnancy in general gynaecologists practices and specialised centres for prenatal care in Germany
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	146 women (including 11 with Down's syndrome) excluded as results could not be assigned to gynaecologists or prenatal centre group.

Krantz 2000

Clinical features and settings	Routine screening.
Participants	10,251 participants. USA. September 1995 to June 1998. Pregnant women. Singleton pregnancies. Maternal age 34.7% ≥ 35 years. No diabetes. 9-13 weeks' gestation.
Study design	Prospective cohort..
Target condition and reference standard(s)	Down's syndrome: 50 cases (33 had undergone biochemical testing) Reference standard: not reported.
Index and comparator tests	Maternal age. Dried blood samples tested for: First trimester NT in 5809 patients (FMF methods). First trimester free ßhCG and PAPP-A in 10,251 patients (enzyme-linked immunosorbent assay procedures)
Follow-up	No details of follow-up reported.
Aim of study	To assess the effectiveness of free ßhCG, PAPP-A and NT for first-trimester screening for Down's syndrome and trisomy 18
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear reference standard.
Partial verification avoided? All tests	Unclear	Unclear if all patients had a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if choice of reference depended on index test results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

Krantz 2000 (*Continued*)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Kratzer 1991

Clinical features and settings	High-risk referral for invasive testing.
Participants	141 participants. USA. Dates not stated. Pregnant women. Controls matched for maternal age. Singleton pregnancies. 9-12 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 17 cases Reference standard: CVS
Index and comparator tests	Frozen serum samples tested for: First trimester hCG and free ßhCG (double antibody radio-immunoassay) First trimester free αhCG (radio-immunoassay, monoclonal antibody, Biomerica Inc) First trimester progesterone (radio-immunoassay).
Follow-up	100% karyotyping.
Aim of study	To present evidence on the value of first trimester serum assays as an early, non-invasive screen for aneuploidy
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description

Kratzer 1991 (*Continued*)

Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index tests interpreted without knowledge of reference standard results (index tests conducted blind to outcome)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Laigaard 2003

Clinical features and settings	Routine screening.
Participants	172 participants (18 cases of Down's syndrome and 154 controls) Denmark - University hosp1T ITAl. Dates not specified. Pregnant women. Singleton pregnancies. 8-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 18 cases. Reference standards: karyotyping, unclear reference standard for controls

Laigaard 2003 (*Continued*)

Index and comparator tests	Frozen serum tested for: First trimester ADAM12 (ELISA, 6E6 and 8F8 antibodies).
Follow-up	No details of follow-up reported.
Aim of study	To determine whether ADAM12 concentration is a useful indicator of fetal health
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear reference standard in controls.
Partial verification avoided? All tests	Unclear	Unclear if all women had a reference standard.
Differential verification avoided? All tests	No	Different reference standards used.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Macintosh 1993

Clinical features and settings	High-risk referral for invasive testing.
Participants	692 participants. UK and Italy. Dates not specified. Pregnant women. Median maternal age 38 years (27-40 years). Singleton pregnancies. 6-12 weeks' gestation.
Study design	Retrospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 14 cases. Reference standard: CVS.
Index and comparator tests	Maternal age. Frozen serum tested for: First trimester serum SP1 (Radioimmunoassay).
Follow-up	100% karyotyping.
Aim of study	To examine the relationship between first trimester maternal serum SP1 and the karyotype of the pregnancy and to quantify its potential use as a screening test
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results

Macintosh 1993 (*Continued*)

Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Muller 2003a

Clinical features and settings	Routine screening.
Participants	5694 pregnant women who had first trimester NT and biochemical testing France - 9 centres serving 12 maternity units. January 1998-June 2001. Singleton pregnancies. Maternal age not reported. 11-13 weeks' gestation.
Study design	Retrospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 26 cases. Reference standards: invasive testing (offered to women with high NT measurement) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT in 98% of patients (methods not specified. 60 sonographers - 2 trained by FMF, who trained 30 in turn. 8 externally trained in France. 20 were self-taught. Machines not specified) Frozen serum tested for: First trimester PAPP-A (99% of patients), free ßhCG 99% of patients and AFP (93% of patients) (time-resolved fluorescent assay, Perkin-Elmer Life sciences) Risk cut-point 1:250.
Follow-up	Data from the French national screening programme used for follow-up at birth. 211 women (3.7%) who did not return after NT or were found to be > 14 weeks were excluded. It is unclear how many patients had follow-up to birth
Aim of study	Prospective study of NT and retrospective evaluation of serum (in same patient population) to evaluate whether or not to move the national French Down's screening programme to a first trimester programme
Notes	FMF methods - some self-taught sonographers.

Muller 2003a (*Continued*)

Table of Methodological Quality		
Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice .
Uninterpretable results reported? All tests	Yes	Women with NT too small to measure assumed to have NT of < 0.5 mm
Withdrawals explained? All tests	Yes	Women failing to return or who more than 14 weeks' pregnant were excluded (214)

Nebiolo 1990

Clinical features and settings	High-risk referral for invasive testing.
Participants	492 participants. Italy. Dates not specified. Pregnant women, approximately 75% were aged ≥ 35 years. Singleton pregnancies. 8-12 weeks' gestation.
Study design	Retrospective cohort study.

Target condition and reference standard(s)	Down's syndrome: 9 cases. Reference standard: CVS.
Index and comparator tests	Frozen serum tested for: First trimester serum AFP and beta/alpha hCG ratio (simultaneous sandwich monoclonal based radioimmunoassay)
Follow-up	100% karyotyping.
Aim of study	To determine the efficacy of combined maternal serum AFP and hCG screening in detecting chromosome defects in the first trimester of pregnancy
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical tests conducted blind to pregnancy outcome)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	Yes	Samples from 48 patients were either no longer available or had been stored at 4^{o}C and were discarded.

Withdrawals explained? All tests	No	No details of withdrawals given.

Niemimaa 2001a

Clinical features and settings	Routine screening.
Participants	2515 participants. Finland - primary care centres and maternity clinics of hospitals During 1999. Pregnant women, 17.5% ≥ 35 years. 10-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 8 cases. Reference standards: invasive testing (patients considered high risk based on NT screening) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (≥ 3 mm) (64% of women) (method not described) Fresh serum tested for: First trimester free ßhCG and PAPP-A (Wallac analytes and 1st trimester risk calculation programme Maternal weight correction) Risk cut-point 1:250.
Follow-up	Follow-up data from maternity clinics and the National Research and Development Centre for Welfare and Health. Test negative patients followed up by contacting all maternity clinics and the National Research and Development Centre for Welfare and Health. Unclear if follow-up information was obtained in all cases
Aim of study	To evaluate efficacy of combining first trimester maternal serum and fetal NT measurement in screening for Down's syndrome in Finland
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.

Niemimaa 2001a (*Continued*)

Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Noble 1995

Clinical features and settings	Routine screening in a high-risk population.
Participants	2529 participants. UK. October 1994 to April 1995. Singleton pregnancies. Pregnant women. Median maternal age 34 years (15-47 years), 47% $\geq$ 35 years. 10-14 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 61 cases. Reference standards: karyotyping performed (27% of women) due to increased NT (14%), advanced maternal age (10%), previous chromosomally abnormal child (0.5%) or parental anxiety (2%). Ultrasound examination at 20 weeks (65% of patients). Follow-up to birth (9% of women)
Index and comparator tests	Maternal age. First trimester NT (methods not stated). Fresh serum (or serum frozen over a weekend) tested for: First trimester free ßhCG (immunoradiometric assay, CIS).
Follow-up	Pregnancy outcome obtained from maternity units or the patients themselves. Follow-up information only appears to have been obtained in 9% of cases (second trimester

Noble 1995 (*Continued*)

	ultrasound used as reference standard for other women)
Aim of study	To measure the contribution of maternal serum free ßhCG in a screening programme for fetal trisomy 21 based on fetal NT in the first trimester of pregnancy
Notes	No proper results, data are presented for this study.

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	No	Invasive testing, ultrasound at 20 weeks or follow-up to birth
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Noble 1997

Clinical features and settings	Routine screening, women self-referred for first trimester NT
Participants	876 participants. UK - Research Centre for Fetal Medicine. Dates not stated.

	76 cases of Down's syndrome. 800 controls matched for maternal and gestational age. Pregnant women. Median maternal age 34 years (15-47 years). Singleton pregnancies. 10-14 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 76 cases. Reference standards: CVS, follow-up to birth not reported.
Index and comparator tests	Maternal age. Frozem serum tested for: First trimester serum Inhibin A (ELISA). Fresh serum (or serum stored over weekend) tested for: First trimester serum free ßhCG (immunoradiometric assay, CIS France)
Follow-up	Details of methods of follow-up not reported.
Aim of study	To determine the relationship between maternal serum first trimester Inhibin A and free ßhCG concentrations in chromosomally normal pregnancies and to compare 2 biochemical markers for their sensitivity in identifying trisomy 21 pregnancies
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear what the reference standard was.
Partial verification avoided? All tests	Unclear	Unclear if all women had a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if the reference standard differed between women.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results

Noble 1997 (*Continued*)

Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical tests conducted blind to pregnancy outcome).
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

O'Leary 2006

Clinical features and settings	Routine screening.
Participants	22,340 participants. Australia - 13 ultrasound practices. August 2001 to October 2003. Singleton pregnancies. Pregnant women aged 14-47 years (median 31 years), 20% ≥ 35 years 11-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 60 cases. Reference standards: CVS or amniocentesis (women assessed to be high risk on screening) , or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (FMF methods). First trimester free ßhCG and PAPP-A (machine not stated). All study participants underwent all tests. Risk cut-point 1:300.
Follow-up	Follow-up data obtained by review of the Midwives Notification System and the Birth Defects Registry. 415 patients (1.8%) excluded due to no follow-up data. Patients with multiple pregnancies or incomplete screens (n = 3946) were also excluded from the study
Aim of study	To assess fetal outcomes for pregnancies identified at increase risk for Down's syndrome by first trimester combined ultrasound examination and maternal serum biochemistry
Notes	Appears likely that patients with miscarriages and terminations excluded

Table of Methodological Quality

O'Leary 2006 (*Continued*)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Unclear	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	Yes	Details given of patients excluded due to incomplete screening data or loss to follow-up

Orlandi 1997

Clinical features and settings	Routine screening of general- and high-risk women.
Participants	2,010 participants (744 in subgroup undergoing NT testing). Italy. Dates not reported. Recruited through private physician or genetic counselling program for women of advanced maternal age Pregnant women aged 15-46 years, 35% > 35 years. Singleton pregnancies. 9-13 weeks' gestation.
Study design	Cohort.

Target condition and reference standard(s)	Down's syndrome: 11 cases (7 in subgroup with NT testing). Reference standards: not reported.
Index and comparator tests	Maternal age. First trimester NT (37% of patients) (FMF methods, Toshiba SSA 250A or Acuson XP 10) First trimester free ßhCG and PAPP-A (all patients) (dried blood samples, enzyme-linked immunosorbent assays) Risk cut-point 1:380.
Follow-up	Not reported.
Aim of study	To evaluate first trimester combined screening for Down's syndrome
Notes	Unclear as to what reference standard (if any) was used. All cases of Down's syndrome identified had been picked up by screening

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Reference standard not reported.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if the choice of reference standard depended on screening results
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements

Orlandi 1997 (*Continued*)

Withdrawals explained? All tests	Yes	Details given of women undergoing NT but not biochemical testing

Palomaki 2007

Clinical features and settings	Routine screening.
Participants	10,775 participants. Canada - General Hospital. October 2003-November 2004. Pregnant women. Mean maternal age 32.3 years (SD 4.6 years). 10-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 23 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (encouraged to only accept measurements from sonographers with FMF certification) First trimester PAPP-A (AutoDELFIA, PerkinElmer). First trimester hyperglycosylated-hCG (Nichols Advantage Specialty system, Nochols Institute Diagnosics)
Follow-up	From electronic record searches of local patient and cytogenetic records and case finding of local and regional birth records
Aim of study	To validate Down's syndrome screening protocols that include hyperglycosylated-hCG measurements
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.

Palomaki 2007 (*Continued*)

Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Qin 1997

Clinical features and settings	Routine screening.
Participants	702 participants. Copenhagen. Dates not specified. Pregnant women. 156 cases of Down's syndrome (25 in weeks 3-9 and 131 in weeks 10-20 gestation) 546 controls (260 in weeks 3-9 and 286 in weeks 10-20 gestation) 5-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 25 cases (3-9 weeks' gestation). Reference standards: CVS, amniocentesis, karyotyping at birth, unclear reference standard for controls
Index and comparator tests	Frozen serum tested for: First trimester schwangerschaftsprotein 1 (SP1) (non-competitive time-resolved immunofluorometric assay, A131, DAKO A/S))
Follow-up	No details of follow-up reported.
Aim of study	To assess the potential of the maternal concentration of schwangerschaftsprotein 1 as a marker for Down's syndrome pregnancies

Qin 1997 (*Continued*)

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Sahota 2010

Clinical features and settings	Routine screening.
Participants	10,854 pregnancies with complete outcome data. China - University Hospital. January 2005-May 2008. Pregnant women. Singleton pregnancies. Median maternal age 33.1 years, 30.1% of women aged ≥ 35 years 10-13 weeks' gestation.
Study design	Retrospective cohort.

Target condition and reference standard(s)	Down's syndrome: 32 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF accredited sonographers, HDI 5000, Philips Medical System) First trimester PAPP-A and free ßhCG (kryptor analyser, Brahms Diagnostica GmbH)
Follow-up	Fetal karyotypes were entered into a database when information was available. Data on pregnancy outcomes were obtained from either a local maternity database (for those who delivered in the unit) or via telephone calls to patients
Aim of study	To assess the relative performance of a multi-stage first trimester screening protocol for fetal Down's syndrome
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Schaelike 2009

Clinical features and settings	Routine screening.
Participants	10,668 participants with complete outcome data. Germany - Private centre. Pregnant women. November 2000-December 2006. Singleton pregnancies. Maternal age ≥ 35 years in 31.0% of women. 11-13 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 59 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF certified physicians). First trimester PAPP-A and free ßhCG (Kryptor analyser, Brahms GmbH) Cut-point 1:300.
Follow-up	Information provided by either obstetric departments or obstetricians. Results obtained from CVS and amniocentesis, as well as karyotypes from aborted fetal tissue or postnatal investigations. 3.9% of women were lost to follow-up and were excluded from the study
Aim of study	To assess the performance of a combined first trimester screening concept for trisomies 21, 18 and 13 applied to a low- and high-risk patient sample in a specialised private centre for prenatal medicine.
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Scott 2004

Clinical features and settings	Routine screening.
Participants	2053 participants. Australia - Private practice (Sydney Ultrasound for Women). July 2000 to May 2002. Pregnant women 15-44 years (median 32 years). Singleton pregnancies. 11-14 weeks' gestation.
Study design	Prospective cohort study.
Target condition and reference standard(s)	Down's syndrome: 5 cases. Reference standards: invasive testing or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (FMF methods, sagittal plane, ATL 5000; Philips) First trimester free ßhCG and PAPP-A (kryptor analyser, Brahms Diagnostics) All participants had all tests. Risk cut-point 1:300.
Follow-up	Data obtained from referring doctors or patients via letter, phone or completed feedback form given at the time of consultation. Only cases of known outcome included in the study. 68 (1.3%) lost to follow-up, largely due to miscarriage (n = 20) and loss to follow-up (n = 40)
Aim of study	To report the sensitivity of combined first trimester biochemistry and ultrasound screening for Down's syndrome in an Australian private practice specialising in obstetric ultrasound
Notes	Only women having biochemical testing before NT were included in the study. This was done to avoid bias from women declining biochemical testing following negative NT

Scott 2004 (*Continued*)

Table of Methodological Quality		
Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	No details of withdrawals given.

Spencer 1999a

Clinical features and settings	Women referred for invasive testing or self-referred for screening
Participants	1156 participants. UK - Fetal medicine research centre. Dates not specified. 210 cases of Down's syndrome, maternal age 19-46 (median 38 years) 946 controls matched for gestational and maternal age, maternal age 15-47 years (median age 36 years) 10-14 weeks' gestation.
Study design	Case-control study.

Spencer 1999a (*Continued*)

Target condition and reference standard(s)	Down's syndrome: 210 cases. Reference standards: invasive testing (high-risk women) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (methods not reported). First trimester free ßhCG and PAPP-A (Kryptor analyser, time resolved amplified cryptate emission (TRACE))
Follow-up	Details of methods for follow-up to birth not reported.
Aim of study	To examine the potential impact of combining maternal age with fetal NT thickness and maternal serum free ßhCG and PAPP-A in screening for trisomy 21 at 10-14 weeks of gestation
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.

Spencer 1999a (*Continued*)

Withdrawals explained? All tests	No	No details of withdrawals given.

Spencer 2002a

Clinical features and settings	Routine screening.
Participants	278 participants. UK - Single hosp1T ITAl study (OSCAR screening program). Samples collected since 1998. 54 cases of Down's syndrome, maternal age 20-44 years, median 36 years 224 controls (no details of selection), maternal age16-41 years, median 30 years 11-13 weeks' gestation..
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 54 cases. Reference standards: no description given.
Index and comparator tests	Maternal age. First trimester NT (FMF methods). First trimester free ßhCG, PAPP-A and ThCG (Kryptor Analyser (TRACE) and automated immunofluorescent assays) All women underwent all tests.
Follow-up	Methods for follow-up to birth not reported.
Aim of study	To assess serum hyperglycosylated hCG for use in the first trimester of pregnancy as a marker of Down's syndrome
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth (Nicolaides ref (OSCAR)).
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.

Spencer 2002a (*Continued*)

Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear of all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Torring 2010

Clinical features and settings	Routine screening.
Participants	691 participants: 46 cases and 645 controls. Denmark - nationwide screening programme. Dates not reported. Pregnant women. Singleton pregnancies. Mean maternal age cases 35 years, controls 31 years. 8-11 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 46 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (11-13 weeks' gestation) (FMF certified sonographers) Fresh serum tested for: First trimester PAPP-A and free ßhCG (8-11 weeks' gestation) (Kryptor analyser, Brahms) Frozen serum tested for: First trimester ADAM12s (8-11 weeks' gestation) (Kyptor analyser, assay by Cezanne SAS, TRACE technology)
Follow-up	Details not reported.
Aim of study	To determine whether ADAM12s is a useful serum marker for fetal trisomy 21 using the mixture model

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Tsukerman 1999

Clinical features and settings	Routine screening.
Participants	1595 participants. Belarus. Started January 1996. Pregnant women. 1,564 controls matched for gestational age and duration of storage 8-13 weeks' gestation.
Study design	Case-control study.

Target condition and reference standard(s)	Down's syndrome: 31 cases. Reference standards: karyotyping, karyotyping at birth, follow-up to birth not reported
Index and comparator tests	Frozen or fresh serum tested for: First trimester free ßhCG, AFP and PAPP-A (DELFIA, EG&G Wallac Oy)
Follow-up	No details of follow-up reported.
Aim of study	To report results of a large population study looking at AFP, free ßhCG and PAPP-A in the first trimester of pregnancy among women having routine ultrasound dating as part of NT assessment
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Women received different reference standards.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of some index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice.
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Valinen 2007

Clinical features and settings	Routine screening
Participants	7534 participants. Finland - screening programme. 2002-2004. Pregnant women. Singleton pregnancies. Mean maternal age 29.6 years, 18.6% ≥ 35 years. 10-12 weeks' gestation.
Study design	Retrospective cohort.
Target condition and reference standard(s)	Down's syndrome: 30 cases (24 underwent NT as well as biochemical testing) Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. First trimester NT (trained nurses, midwives and doctors) (4765 women) First trimester PAPP-A and free ßhCG (details not reported) (7534 women) Cut-point 1:250.
Follow-up	Contacted chromosome laboratory at the department of clinical genetics in the Oulu university clinic and the Finish Register of Congenital Malformation and the National Research and Development Centre for Welfare and Health
Aim of study	To compare the efficacy of both separate and combined maternal serum testing and fetal NT measurement in the first trimester screening for Down's syndrome in northern Finland
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Valinen 2009

Clinical features and settings	Routine screening.
Participants	279 participants: 53 cases and 226 controls matched for maternal and gestational age and sample storage time Finland - screening programme. May 2002-December 2007. Pregnant women. Maternal age not reported. 9-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 53 cases (in 5 cases, NT not measured). Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. Fresh serum tested for: First trimester PAPP-A and free ßhCG (details not reported). Frozen serum tested for: First trimester ADAM12 (DELFIA/AutoDELFIA ADAM12 research kit, PerkinElmer Wallac) Cut-point 1:250.
Follow-up	Details not reported.
Aim of study	To investigate whether incorporating the measurement of ADAM12 in the risk calculation program LifeCycle can improve Down's syndrome screening in the first trimester
Notes	

Table of Methodological Quality

Valinen 2009 (*Continued*)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Van Lith 1992

Clinical features and settings	High-risk referral for invasive testing.
Participants	1372 participants (24 cases and 1348 controls, criteria for matching not reported) The Netherlands - 6 prenatal diagnostic centres. Dates not stated. Pregnant women. Less than 13 weeks' gestation.
Study design	Case-control study (changed from cohort).
Target condition and reference standard(s)	Down's syndrome: 24 cases. Reference standard: CVS.
Index and comparator tests	Frozen serum rested for: Total hCG (IMx hCG assay, Abbott).

Van Lith 1992 (*Continued*)

Follow-up	100% karyotyping.
Aim of study	To assess the value of MS-hCG in the first trimester of pregnancy in screening for Down's syndrome
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.
Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results (biochemical measurement conducted blind to pregnancy outcome)
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

Wald 2003a

Clinical features and settings	Routine screening.
Participants	606 participants. UK and Austria - multicentre trial. September 1996 to April 2000. Pregnant women: 101 cases, 505 controls matched for gestation, duration of storage and centre 9-13 and 14-20 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 101 cases. Reference standards: invasive testing (following second trimester screening) or follow-up to birth
Index and comparator tests	First trimester NT (midsagittal section, optimal magnification of thickness of translucent space between inner skin surface and fascia covering cervical spine (white black interface (outer) - black white interface (inner), 41 models of ultrasound machine, 20 minutes allotted scanning time) First and second trimester serum AFP, hCG, uE3, PAPP-A, free ßhCG (time resolved fluoroimmunoassay, AutoDELFIA) First and second trimester inhibin A (Sandwich enzyme linked immunosorbent assay, Oxford bioinnovation) First and second trimester urinary beta core fragment, total hCG, ITA and free ßhCG (ITA and beta core fragment, Quest diagnostics USA)
Follow-up	Follow-up by: 1) Staff at local hospitals completed a study outcome form at, or just after delivery, 2) Study records of CVS, amniocentesis or karyotype at birth linked to information from cytogenic laboratories, 3) Study records linked to records of cases of Down's syndrome from the National Down's Syndrome Cytogenetic Register, 4) Information obtained from local obstetrical outcome records, 5) Forms sent to all women with a request to return details of the outcome of their pregnancy, 6) individual searches in respect of women whose outcomes of pregnancy had not been obtained by any of the previous methods. 96% birth/karyotype full outcome documentation obtained
Aim of study	To identify the most effective, safe and cost-effective strategy for antenatal screening for Down's syndrome using NT, maternal serum and urine markers in the first and second trimesters of pregnancy and maternal age in various combinations
Notes	Performance of screening assessed at 17 weeks' gestation. Study tried to be non-interventional in the first trimester - second trimester testing was aimed to be used as the basis for any referral for invasive testing

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.

Wald 2003a (*Continued*)

Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	Rates of NT failure on average 9%. pre-10 weeks' gestation, > 33% failure rate, declined to 7% at 12 weeks
Withdrawals explained? All tests	No	No details of withdrawals given.

Wallace 1995

Clinical features and settings	Routine screening.
Participants	112 participants. UK. Dates not stated. Pregnant women. 23 cases (maternal age 22-44 years, mean 32 years). 89 controls matched for gestational age and duration of sample storage (maternal age 19-38 years, mean 28 years) 11-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 23 cases. Reference standard: not reported.
Index and comparator tests	Frozen serum tested for dimeric first trimester Inhibin A (enzyme-linked two-site immunoassay)

Follow-up	Methods of follow-up not reported.
Aim of study	To evaluate dimeric first trimester inhibin A as a possible first trimester screening marker for Down's syndrome screening
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Unclear	Unclear reference standard.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	Unclear	Unclear if the reference standard differed between women.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Wapner 2003

Clinical features and settings	Routine screening.
Participants	8216 participants. USA - multicentre study (12 prenatal diagnostic centres). Dates not specified. Singleton pregnancies.

	Pregnant women with mean age 35 years (SD 4.6), 50% ≥ 35 years 11 to 14 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 61 cases. Reference standards: invasive testing, miscarriage with cytogenetic testing, follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (FMF methods). Dried blood samples tested for: First trimester free ßhCG and PAPP-A (dried blood samples, enzyme-linked immunoadsorbent assay as previously described) Risk cut-point 1:270.
Follow-up	Follow-up to birth by directly following up women and reviewing delivery records. An effort was also made to obtain information on terminated or miscarried pregnancies. 196 (2.3%) of patients without follow-up information were excluded
Aim of study	To evaluate the use of combined first trimester markers for aneuploidy in clinical practice
Notes	16 live Down's syndrome births.

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results

Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	Yes	No details of withdrawals given.

Weinans 2005

Clinical features and settings	High-risk referral for invasive testing.
Participants	344 participants. The Netherlands - antenatal diagnosis unit. 1999-2002. Pregnant women with mean age 38 years (SD 2.7 years) for cases and 37 years (SD 3.0) for controls 24 cases, 320 controls matched for maternal and gestational age and length of storage Singleton pregnancies. 9 to 11 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 24 cases. Reference standard; CVS.
Index and comparator tests	Maternal age. Frozen serum samples tested for: First trimester serum free ßhCG and PAPP-A (fluoroimmunoassay, Auto Delfia, Perkin Elmer) First trimester maternal serum ITA (immunochemiluminometric assay, Nichols Advantage platform)
Follow-up	100% karyotyping.
Aim of study	To investigate Down's syndrome screening performance of serum ITA before 12 weeks' gestation and compare it with performance of PAPP-A and free ßhCG in the same sample set
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Selective testing of high-risk women as done in practice.

Weinans 2005 (*Continued*)

Acceptable reference standard? All tests	Yes	CVS.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	Yes	All women received the same reference standard.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	Yes	Reference standard interpreted without knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements.
Withdrawals explained? All tests	No	No details of withdrawals given.

Wojdemann 2005

Clinical features and settings	Referrals for screening.
Participants	8622 participants (6441 with serum screening). Denmark - 3 obstetrics departments. March 1998 to June 2001 Pregnant women with mean age 29 years, 10.8% ≥ 35 years. Singleton pregnancies. 11 to 14 weeks' gestation.
Study design	Prospective cohort.
Target condition and reference standard(s)	Down's syndrome: 12 cases. Reference standards: invasive testing (in cases of increased risk) or follow-up to birth
Index and comparator tests	Maternal age. First trimester NT (all patients) (FMF methods, Logic 700 MR machine) First trimester free ßhCG (AFP/ßhCG Auto Delfia kit) and PAPP-A (In-house ELISA (Sandwich)) in 6,441 patients (75%) Risk cut-point 1:250.

Follow-up	Cross-checking with all the chromosome laboratories in Denmark. Follow-up in 96.2% of pregnancies through patients records
Aim of study	To determine the performance of screening for Down's syndrome and other major chromosomal abnormalities using NT, free ßhCG and PAPP-A in a prospective study of a non-selected population
Notes	Uptake of screening was 73% (9941 accepted out of 13,621 offered screening) Women with miscarriages excluded from the study. 3 live Down's syndrome births.

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Unclear	Unclear if all women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.
Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Yes	Index test interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	Yes	NT could not be measured in 2.5% of cases.
Withdrawals explained? All tests	No	No details of withdrawals given.

Zaragoza 2009

Clinical features and settings	Routine screening.
Participants	699 participants: 90 cases and 609 controls matched for length of storage UK - single centre. Dates not reported. Pregnant women. Singleton pregnancies. Median maternal age cases 37.9 years (19.1-46.5 years), controls 32.7 years (16.1-45.2 years) 11-13 weeks' gestation.
Study design	Case-control study.
Target condition and reference standard(s)	Down's syndrome: 90 cases. Reference standards: karyotyping or follow-up to birth.
Index and comparator tests	Maternal age. Fresh samples tested for: First trimester PAPP-A and free ßhCG (Delfia Express system, PerkinElmer, Waltham) Frozen samples tested for: First trimester PlGF (ELISA, Quantikine human PlGF immunoassay, R&D systems Europe Ltd)
Follow-up	Karyotype results and details on pregnancy outcome were added to database as soon as they became available
Aim of study	To investigate the potential value of maternal serum placental growth factor (PlGF) in first trimester screening from trisomy 21 and other major chromosomal abnormalities
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Routine screening of typical pregnant population.
Acceptable reference standard? All tests	Yes	Karyotyping or follow-up to birth.
Partial verification avoided? All tests	Yes	All women received a reference standard.
Differential verification avoided? All tests	No	Choice of reference standard depended on index test results.
Incorporation avoided? All tests	Yes	Reference standard was independent of the index test.

Zaragoza 2009 (*Continued*)

Reference standard results blinded? All tests	No	Reference standard interpreted with knowledge of index test results
Index test results blinded? All tests	Unclear	Unclear if all index tests interpreted without knowledge of reference standard results
Relevant clinical information? All tests	Yes	Information available as would be in standard clinical practice
Uninterpretable results reported? All tests	No	No details given for test failures/uninterpretable measurements
Withdrawals explained? All tests	No	No details of withdrawals given.

AFP: alpha-fetoprotein
αhCG: alpha human chorionic gonadotrophin
ßhCG: beta human chorionic gonadotrophin
CVS: chorionic villus sampling
ELISA: enxyme-linked immunosorbent assay
FMF: Fetal Medicine Foundation
GHBP: growth hormone binding protein
hCG: human chorionic gonadotrophin
ITA: invasive trophoblast antigen
IQR: interquartile range
NT**:** nuchal translucency
PAPP-A: Pregnancy-associated plasma protein A
PGH: placental growth hormone
PIGF: placental growth factor
PROMBP: proform of eosinophil major basic protein
SD: standard deviation
SP 1: Schwangerschafts protein 1
uE3: unconjugated oestriol

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Abbas 1995	Unable to extract useful data.
Abdul-Hamid 2004	No Down's syndrome pregnancies.
Abraha 1999	Unable to extract useful data.
Abu-Rustum 2010	Not Down's syndrome specific.

(*Continued*)

Achiron 2010	Study only includes cases of Down's syndrome.
Adekunle 1999	Unable to extract useful information.
Agaard-Tillery 2010	Results presented in another study.
Aitken 1993	Unable to extract useful data.
Aitken 1996a	Fewer than 80% of pregnancies had gestational age confirmed by USS
Aitken 1996b	Fewer than 80% of pregnancies had gestational age confirmed by USS
Ajayi 2011	No diagnostic data.
Akbas 2001	Less than 5 Down's syndrome pregnancies.
Alexioy 2009	Study only includes test positives.
Allingham-Hawkins 2011	Quantitative fluorescent polymerase chain reaction study.
American College 2009	Discussion article.
Antona 1998	Likely fewer than 80% of pregnancies dated by USS.
Antsaklis 1999	Women screened at greater than 24 weeks' gestation.
Anuwutnavin 2009	Second trimester ultrasound.
Ashwood 1987	Unable to extract useful data.
Asrani 2005	Review article.
Audibert 2001	Unable to ascertain whether part of screening population in Rozenberg et al. No response from authors, therefore excluded to reduce risk of data replication
Axt-Fleidner 2006	Unable to extract useful data.
Azuma 2002	Unable to extract useful data.
Baghagho 2004	Unable to obtain paper.
Bahado-Singh 1995	USS markers greater than 14 weeks' gestation.
Bahado-Singh 1996	USS markers greater than 14 weeks' gestation.
Bahado-Singh 1999	USS markers greater than 14 weeks' gestation.

(*Continued*)

Bahado-Singh 2002	USS markers greater than 14 weeks' gestation.
Bahado-Singh 2003	Review article.
Ball 2007	Data from the FASTER trial.
Bar-Hava 2001	No Down's pregnancies in study population.
Barkai 1996	No Down's pregnancies in study population.
Barnabei 1995	No Down's pregnancies in study population.
Bartels 1988	Unable to extract useful data.
Bartels 1993	No Down's pregnancies in study population.
Barth 1991	Second trimester ultrasound study.
Bas-Budecka 2007	No diagnostic data.
Baviera 2004	Unclear method of confirmation of gestational age.
Bazzett 1998	Male versus female fetuses.
Beke 2008	Results are not specific to Down's syndrome.
Bellver 2005	No Down's syndrome pregnancies in study.
Benn 1995	Less than 80% follow-up.
Benn 1996	Less than 80% follow-up.
Benn 1997	No Down's pregnancies in study population.
Benn 1998	Less than 80% follow-up.
Benn 2001	Statistical modelling (computer simulation).
Benn 2002	Modelled data.
Benn 2003a	Less than 80% of pregnancies dated by USS.
Benn 2003b	Editorial.
Benn 2005a	No Down's pregnancies included.
Benn 2005b	Mathematical model.

(*Continued*)

Benn 2007	No follow-up information.
Berry 1995	Less than 80% of pregnancies USS dated.
Berry 1997	Less than 80% of pregnancies USS dated.
Bersinger 1994	Gestational age not USS estimated.
Bersinger 2000	Unable to extract useful data.
Bersinger 2001	No Down's syndrome pregnancies in study population.
Bersinger 2003	Unable to extract useful data.
Bersinger 2004	No Down's syndrome pregnancies in study population.
Bersinger 2005	No Down's syndrome pregnancies in study population.
Bestwick 2008	All healthy pregnancies.
Biggio 2004	Cost-effectiveness analysis.
Bilardo 2011	Not a proper sample - most had elevated NT.
Bindra 2002	Review article.
Blundell 1999	Unable to extract useful data.
Boormans 2010	Study of testing on amniocentesis samples.
Boots 1989	Population risk factor calculations.
Bornstein 2009a	No diagnostic data.
Bornstein 2009b	No diagnostic data.
Bornstein 2010	No diagnostic data.
Borowski 2007	No diagnostic data.
Borrell 2007	No follow-up data.
Borruto 2002	Unable to extract useful data.
Bottalico 2009	Second trimester ultrasound.
Boue 1990	Review article.

(*Continued*)

Bradley 1994	Screen negative population gestations not confirmed by ultrasound
Braithwaite 1996	Review article.
Brambati 1995	USS screening inclusive of women greater than 14 weeks' gestation
Brambati 1996	Review article.
Brizot 1995a	Unable to extract useful data.
Brizot 1995b	Unable to extract useful data.
Brizzi 1989	Second trimester ultrasound.
Brock 1990	Unable to extract useful data.
Calda 2010	No data for false positive rates.
Campogrande 2001	Unable to extract useful data.
Canick 1988	Unable to extract useful data.
Canick 1995	Unable to extract useful data.
Canini 2002	No Down's syndrome pregnancies in study population.
Cans 1998	Second trimester ultrasound.
Carreras 1991	Second trimester ultrasound.
Caughey 2007	No diagnostic data.
Cebesoy 2008	No diagnostic data.
Chelli 2008	No follow-up for false negatives.
Chen 1999	Review article.
Chen 2002	No Down's syndrome pregnancies in study population.
Chen 2004	Less than 5 Down's cases in study population.
Chen 2005	Unable to extract useful data.
Chen 2008	No diagnostic data.
Cheng 1993	Likely that fewer than 80% of gestational age confirmed by USS

(*Continued*)

Cheng 1999	Case series. No Down's syndrome pregnancies in study population
Cheng 2004a	No Down's syndrome pregnancies in study population.
Cheng 2004b	No Down's syndrome pregnancies in study population.
Chitayat 2002	Less than 5 Down's cases in study population.
Chiu 2011	Study of maternal DNA testing.
Cho 2009	Study of testing amniotic fluid.
Chou 2009	Not possible to calculate specificity.
Christiansen 2002	Unable to extract useful data.
Christiansen 2007b	Unable to extract useful data.
Christiansen 2008	No diagnostic data.
Chung 2000	Less than 5 Down's syndrome pregnancies in study population.
CNGOF 1996	Unable to obtain translation.
Cole 1996	Review article.
Comas 2001	USS at greater than 14 weeks.
Comas 2002a	USS at greater than 14 weeks.
Comas 2002b	USS at greater than 14 weeks.
Comstock 2006	Unable to extract useful data.
Conde 1998	Review article.
Cowans 2011	No diagnostic data.
Crossley 1991	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1993	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1996	No Down's syndrome pregnancies in study population.
Crossley 2002b	Adjustment factors for smokers.
Cuckle 1984	Gestational age not confirmed by USS.

(*Continued*)

Cuckle 1987a	Gestational age not confirmed by USS.
Cuckle 1987b	No gestational age limits given.
Cuckle 1990	Paper presenting adjustment factors.
Cuckle 1996	Data modelled on 4 meta-analysed studies.
Cuckle 1999a	Unable to extract useful data.
Cuckle 1999b	Review article.
Cullen 1990	Abnormal scans only in study population.
Cusick 2004	Less than 5 Down's syndrome pregnancies in study population.
Cusick 2007	Second trimester ultrasound.
D'Ottavio 1997	Second trimester USS.
Dancoine 2001	No Down's syndrome pregnancies in study population.
Dane 2008	Not specific to Down's syndrome.
De Biasio 2000	Unable to extract useful information.
De Biasio, 1999	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Biasio, 2001	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Graaf 1991	Unable to extract useful data.
De Graaf 1999b	Modelled data.
Del Carmen Saucedo 2009	No follow-up information.
DeVore 2001	Second trimester ultrasound.
Dhaifalah 2007a	Unable to obtain translation.
Dhaifalah 2007b	Unable to obtain translation.
Dhallan 2007	DNA testing of blood samples from parents.
Dickerson 1994	Comment.

(*Continued*)

Dimaio 1987	Gestational age by USS only in screen-positive population.
Doran 1986	Ultrasound confirmation of gestational age performed in screen positive women only
Dreux 2008	No information for specificity.
Drugan 1996a	Second trimester ultrasound.
Drugan 1996b	Unable to extract useful data.
Drysdale 2002	Fewer than 5 Down's syndrome pregnancies in population.
Dugoff 2008	Not specific to Down's syndrome.
Ebell 1999	Review article.
Economides 1998	Unable to extract useful data.
Erickson 2004	No Down's syndrome pregnancies in population.
Evans 1996	No Down's syndrome pregnancies in population.
Evans 2007	Data previously presented in another study.
Falcon 2005	Unable to extract useful data.
Falcon 2006	Unable to extract useful data.
Ford 1998	Audit.
Frishman 1997	No Down's syndrome pregnancies in population.
Fukada 2000	Unable to extract useful data.
Gaudry 2009	Study of karyotyping.
Gebb 2009	Study only examines screen positives.
Geerts 2008	Study only examines abnormal fetuses.
Geipel 2010	Second trimester ultrasound.
Gekas 2009	Diagnostic data from other studies.
Gekas 2011a	Diagnostic data from other studies.
Gekas 2011b	Diagnostic parameters from other studies.

(*Continued*)

Gerovassili 2007	No diagnostic data.
Ghidini 1998	Comparison of male versus female fetuses.
Goetzinger 2010	Second trimester ultrasound.
Goldie 1995	Fewer than 80% of study population and gestational age confirmed by USS
Gollo 2008	Only one case of Down's syndrome.
Gonçalves 2004	Greater than 14 weeks USS screening.
Goodburn 1994	Likely that fewer than 80% of pregnancies had gestational age estimated by USS
Gorduza 2007	Study of FISH technique.
Grace 2010	Second trimester ultrasound.
Grati 2010	No diagnostic data.
Gray 2009	Second trimester ultrasound.
Gregor 2007	Unable to obtain translation.
Gregor 2009	Unable to obtain translation.
Grether 2009	Systematic review and guidelines.
Grozdea 2002	Unable to extract useful data.
Guo 2010	Study of fetal samples.
Gyselaers 2004a	Less than 80% follow-up.
Gyselaers 2004b	Less than 80% follow-up.
Gyselaers 2006a	Unaffected pregnancies only.
Gyselaers 2006b	Unable to extract useful data.
Hackshaw 1995	No Down's syndrome pregnancies in population.
Hackshaw 2001	No Down's syndrome pregnancies in population
Haddow 1992	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Hadzsiev 2007	Study of FISH technique.

(*Continued*)

Hafner 1995	Less than 5 Down's pregnancies in study population.
Hallahan 1998	Gestational age greater than 24 weeks.
Han 2008	Study of findings on amniocentesis.
Harper 2010	Second trimester ultrasound.
Harrison 2006	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Harry 2006	Editorial.
Hayashi 1995	Unable to extract useful data.
Hayashi 1996	Less than 5 Down's pregnancies in study population.
Heikkila 1997	Fewer than 80% of pregnancies had gestational age confirmed by USS
Heinig 2007	No Down's syndrome data.
Heinonen 1996	No Down's syndrome pregnancies in population.
Herman 2000	No Down's syndrome pregnancies in study population.
Herman 2003	Correlation between markers, not evaluation of screening tests
Herrou 1992	Unable to extract useful data.
Hershey 1985	Gestation unclear.
Hershey 1986	Gestation based on LMP.
Hewitt 1993	Unable to extract useful data.
Hills 2010	Study of testing on CVS and amniocentesis samples.
Ho 2010	Study of FISH diagnosis.
Hogdall 1992	Unclear method of determination of gestational age. Unable to extract useful data
Hong Kong Practitioner	CME.
Hoogendoorn 2008	Diagnostic data from other studies used.
Howe 2000	Second trimester ultrasound scans.
Hsiao 1991	Unable to obtain translation.

(*Continued*)

Hsieh 1999	No Down's syndrome pregnancies in study population.
Hsu 1997	Adjustment factors.
Hsu 1998	No Down's syndrome pregnancies in study population.
Hsu 1999	No Down's pregnancies.
Hu 2007	Same data as Liu 2010.
Huang 2003	No Down's syndrome pregnancies in study population.
Huang 2007a	Not possible to obtain detection rate.
Huang 2007b	No diagnostic data.
Huggon 2004	Study of cardiac function in pregnancies with normal and abnormal NT results
Hui 2003	No Down's syndrome pregnancies in population.
Hui 2005	No Down's syndrome pregnancies in population.
Hultén 2004	Editorial/commentary.
Hung 2003	Modelling.
Hung 2008	Second trimester ultrasound.
Hurley 1993	Unable to extract useful data.
Huttly 2004	No Down's syndrome pregnancies in population.
Hwa 2004	Less than 5 Down's pregnancies in population.
Iles 1996	Review.
Ind 1994	Unable to extract useful data.
Ivorra-Deleuze 2010	No diagnostic data.
Jakobsen 2011	Not Down's syndrome specific.
Jean-Pierre 2005	Review article.
Johnson 1991	Gestational age estimated by USS in fewer than 80% of cases.
Johnson 1993	Normal pregnancies only.

(*Continued*)

Jorgensen 1999	Gestation greater than 14 weeks for USS.
Jorgez 2007	Study of DNA testing on maternal blood.
Josefsson 1998	No Down's syndrome pregnancies in study population.
Jou 2001	Less than 5 Down's syndrome pregnancies in study population.
Jung 2007	Second trimester ultrasound.
Kagan 2006	Screen positive pregnancies only.
Kagan 2007	No diagnostic data.
Kagan 2008	Not Down's syndrome detection.
Kalelioglu 2007	Second trimester ultrasound.
Kautzmann 1995	Fewer than 80% pregnancies had gestational age estimated by USS
Kazerouni 2009	Not possible to obtain complete diagnostic data.
Keith 1992	Summary article.
Kelekci 2004	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995a	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995b	Less than 80% follow-up. Unable to ascertain proportion of population with gestational age confirmed by USS
Kellner 1997	Assumption of normal karyotype without reference standard in significant proportion of control pregnancies
Kirkegaard 2008	False positive rate only calculated for subset of the cohort
Kjaergaard 2008	Unable to obtain translation.
Knight 1990	Review article.
Knight 2001	Validation of a specific assay.
Knight 2005	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Koos 2006	Review article.
Kornman 1996	Less than 5 Down's syndrome pregnancies in population.

(*Continued*)

Kornman 1997	Unable to extract useful information.
Kotaska 2007	No new data.
Kramer 1998	No Down's syndrome pregnancies in study population.
Krantz 1996	Modelled data.
Krantz 2005	Adjustment factor.
Krantz 2007	Uses data from other published studies.
Kulch 1993	No Down's cases in population.
Lai 1998	Modelled population.
Lai 2003	No Down's syndrome pregnancies in study population.
Laigaard 2006a	Unable to extract useful data.
Laigaard 2006b	Simulation.
Lam 1997	Unable to extract useful data.
Lam 1998	Fewer than 80% pregnancies had gestational age estimated by USS
Lam 1999a	No Down's syndrome pregnancies in population.
Lam 1999b	Unable to extract useful data.
Lam 2000	Study of women's decisions about screening.
Lam 2001	Male versus female fetuses.
Lambert-Messerlian 1996	Fewer than 80% of pregnancies USS dated.
Lambert-Messerlian 1998	Unable to extract useful data.
Lauria 2007	No diagnostic data.
Lehavi 2005	Down's syndrome pregnancies only.
Leung 2006	Unable to separate twins from singletons therefore unable to extract useful data
Leymarie 1993	Appears to be a review article (French).
Li 1998	Unable to obtain translation.

(*Continued*)

Li 1999	Unable to obtain translation.
Li 2010	No diagnostic data.
Liao 1997	Unable to obtain translation.
Liao 2001	Unable to extract useful data.
Lim 2002	Second trimester ultrasound.
Lippman 1987	Editorial.
Liu 2003	Unable to obtain translation.
Liu 2010	Not possible to separate out data for cases of Down's syndrome
Lo 2010	Pooled test results.
Lustig 1988	Gestational age by LMP only.
Luthgens 2008	False positive rate and detection rate obtained from different cohorts
MacDonald 1991	Fewer than 80% of gestational ages estimated by USS.
Macintosh 1994	Unable to extract useful data.
Macintosh 1997	Unable to extract useful data.
MacRae 2010	Pooled test results.
Macri 1994	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Macri 1996	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Malone 1998	Review article.
Malone 2003	Review article.
Mandryka-Stankewycz 2009	No diagnostic data.
Mangione 2001	Abnormal screening results only.
Markov 2008	Unable to obtain paper.
Maymon 2001a	No Down's syndrome pregnancies in study population.
Maymon 2001b	No normal test results included therefore unable to extract meaningful data

(*Continued*)

Maymon 2002	No Down's syndrome pregnancies in study population.
Maymon 2004	No Down's syndrome pregnancies in study population.
Maymon 2005	Modelled data.
McDuffie 1996	USS dating on screen positive women only.
Meier 2002	Observed versus expected cases of Down's syndrome in a population
Merkatz 1984	Gestational age not confirmed by ultrasound scan.
Merz 2005	Editorial.
Merz 2008	Data available for only combined ultrasound marker (nuchal translucency) and serum tests
Metzenbauer 2001	Normal pregnancies only.
Metzenbauer 2002	Unable to extract useful data.
Mikic 1999	No Down's syndrome pregnancies in study population.
Miller 1991	Unable to extract useful data.
Milunsky 1989	Fewer than 80% gestational age estimated by USS.
Milunsky 1996	Fewer than 80% gestational age estimated by USS.
Minobe 2002	Gestational age greater than specified limits.
Miron 2008	No diagnostic data.
Miron 2009	No diagnostic data.
Miron 2010	No diagnostic data.
Miyamura 1999	Unable to extract useful data.
Moghadam 1998	Unable to extract useful data.
Monni 2000	Less than 5 Down's syndrome pregnancies.
Monni 2002	Review article.
Mooney 1994	Greater than 24 weeks' gestation.
Muhcu 2008	No diagnostic data.

(*Continued*)

Muller 1994	No Down's syndrome pregnancies in study population.
Muller 1996	Unable to extract useful data.
Muller 1999	Unable to extract useful data.
Muller 2002a	Gestational age greater than 24 weeks.
Muller 2002b	Unable to extract meaningful data - unable to separate double- and triple-test data
Muller 2003b	No Down's syndrome pregnancies in study population.
Murta 2002	Unable to extract useful data.
Musone 2000	Unable to extract useful data.
Musto 1986	Fewer than 80% USS dated.
Myrick 1990	Unable to extract useful data.
Naidoo 2008	Not specific Down's syndrome results.
Nau 2009a	No diagnostic data.
Nau 2009b	No diagnostic data.
Neveux 1996a	No Down's syndrome pregnancies in population.
Neveux 1996b	Unable to extract useful data.
Ng 2004	Unable to extract useful data.
Nicolaides 1992	Study of outcomes of abnormal NT results.
Nicolaides 2000	Review article.
Nicolaides 2004	Review article.
Nicolaides 2005a	Unable to obtain translation - appears to be a review article
Nicolaides 2005b	Unable to obtain translation - appears to be a review article
Nicolaides 2005c	Unable to obtain translation - appears to be a review article
Nicolaides 2005d	Unable to obtain translation - appears to be a review article
Nicolaides 2005e	Unable to obtain translation - appears to be a review article

(*Continued*)

Nicolaides 2005f	Review article.
Niemimaa 2001b	No Down's pregnancies in study population.
Niemimaa 2002	No Down's syndrome pregnancies in population.
Niemimaa 2003	No Down's syndrome pregnancies in population.
Noble 1997a	Unable to extract useful data.
Norgaard 1990	Less than 80% of gestational ages confirmed by USS.
Norton 1992	Unable to extract useful data.
Novakov-Mikic 2007	Out of first trimester screening time frame.
O'Brien 1997a	No Down's syndrome pregnancies in population.
O'Brien 1997b	No Down's syndrome pregnancies in population.
Odibo 2004	Gestational age of greater than 14 weeks in USS population.
Odibo 2007	Second trimester ultrasound.
Odibo 2008	Second trimester ultrasound.
Odibo 2009	No results presented.
Offerdal 2008	Second trimester ultrasound.
Ognibene 1999	Unable to extract useful data.
Oh 2007	No diagnostic data.
Olajide 1989	Unable to extract useful data.
Onda 1996	Unable to extract useful data.
Onda 1998	Unable to extract useful data.
Onda 2000	Less than 80% follow-up.
Orlandi 2002	No Down's syndrome pregnancies in study population.
Ozkaya 2010	Only healthy pregnancies.
Paladini 2007	No diagnostic data.

(*Continued*)

Palka 1998	Twin data used in calculation of the median.
Palomaki 1989	Fewer than 80% USS dated.
Palomaki 1993	No Down's syndrome pregnancies in population.
Palomaki 1994	No Down's syndrome pregnancies in population.
Palomaki 1996	Meta-analysis.
Palomaki 2005	Unable to extract meaningful data.
Panburana 2001	Less than 5 Down's syndrome pregnancies in population.
Pandya 1994	Study of outcomes of abnormal nuchal translucency results.
Pandya 1995	Review article.
Papadopoulou 2008	No diagnostic data.
Parra-Cordero 2007	Second trimester ultrasound.
Paterlini-Brechot 2007	Editorial, no new data.
Paul 2001	Unable to extract useful data.
Peralta 2005	Unable to extract useful data.
Perenc 1998	No Down's syndrome pregnancies in study population.
Perheentupa 2002	No Down's syndrome pregnancies in population.
Perona 1998	Smokers versus non smokers.
Persico 2008	Second trimester ultrasound.
Petervari 2000	Unable to extract useful data.
Petrocik 1989	Likely fewer than 80% USS dated.
Phillips 1992	Gestational age confirmed by USS in less than 80% of population
Phillips 1993	Gestational age confirmed by USS in less than 80% of population
Pihl 2008	Only 2 cases of Down's syndrome.
Pinette 2003	Women screened prior to recruitment.

(*Continued*)

Platt 2004	Unable to extract useful data.
Podobnik 1995	Abnormal results only.
Poon 2009	No diagnostic data.
Prefumo 2002	Comparison of prevalence and prediction.
Prefumo 2004	Comparison of a marker in women of different ethnic origins.
Price 1998	Unable to extract useful data.
Páez 2004	Unable to obtain translation.
Raty 2000	No Down's syndrome pregnancies in population.
Rembouskos 2004	Unable to extract useful data.
Ren 1992	Review article.
Renier 1998	Method of ascertainment of gestational age unclear. Twin gestations included in general population
Resta 1990	Second trimester USS.
Reynders 1997	Fewer than 5 Down's cases.
Reynolds 1989	Explanation of mathematical techniques.
Reynolds 1999	Unable to extract useful data.
Reynolds 2008	Not full diagnostic data.
Ribbert 1996	No Down's syndrome pregnancies in study population.
Rice 2005	Down's syndrome pregnancies excluded from study.
Rich 1991	Unable to extract useful data.
Roberts 1995	No Down's syndrome pregnancies in study population.
Robertson 1991	Editorial.
Rode 2003	No Down's pregnancies.
Ronge 2006	Editorial - summary of FASTER results.
Rose 1995	Review article.

(*Continued*)

Ross 1997	Review article.
Rotmensch 1996	Unable to extract useful data.
Rotmensch 1999	No Down's syndrome pregnancies in study population.
Rozenberg 2006	USS greater than 14 weeks' gestation.
Rudnicka 2002	No Down's syndrome pregnancies in population.
Ryall 1992	Unable to determine method of confirmation of gestational age
Ryall 2001	High-risk results only included (i.e. no screen-negative group for comparison)
Räty 2002	No Down's pregnancies in population.
Sabriá 2002	Unable to ascertain how numbers calculated and from which populations
Sacchini 2003	Unable to extract useful data.
Sahota 2009	No diagnostic data.
Sahota 2010a	Included in Sahota 2010.
Salazar 2007	Unable to obtain paper.
Salazar 2008	Only one case of Down's syndrome.
Saller 1997	Down's syndrome secondary to Robertsonian translocation only. No controls
Salomon 2001	No Down's syndrome pregnancies in population.
Salonen 1997	Fewer than 80% had gestational age estimated by USS.
Saltvedt 2005	Gestation greater than 14 weeks for nuchal scanning.
Saridogan 1996	Down's syndrome and Edward's syndrome affected pregnancies only
Savoldelli 1993	Unable to extract useful data.
Schielen 2009	Full study information not given.
Schiott 2006	Unable to extract useful data.
Schmidt 2007a	Not specific to Down's syndrome.
Schmidt 2007b	No separate Down's syndrome data.

(*Continued*)

Schmidt 2007c	No diagnostic data.
Schmidt 2008a	Not specific to Down's syndrome.
Schmidt 2008b	Not specific to Down's syndrome.
Schmidt 2008c	Not specific to Down's syndrome.
Schmidt 2010	No follow-up data for test negatives.
Schuchter 1998	No Down's pregnancies in study population.
Scott 1995	Less than 5 Down's syndrome pregnancies in study population.
Seeds 1990	Review article.
Seki 1995	No Down's syndrome pregnancies in study population.
Shenhav 2003	No Down's syndrome pregnancies.
Shintaku 1989	Unable to extract useful data.
Shulman 2003	No Down's syndrome pregnancies in population.
Sieroszewski 2008	No Down's syndrome specific information for specificity.
Simon-Bouy 1999	Review article.
Simpson 1986	Gestational age confirmed by USS in less than 80% of population
Smith 1990	Analysis of screen-positive results.
Smith 1996	Review/meta-analysis.
Smith 1999	Unable to extract useful data.
Smith-Bindman 2001	Meta-analysis of second trimester ultrasound markers.
Smith-Bindman 2003	Population study, not examining DTA.
Snijders 1995	Study of prevalence, not screening.
Snijders 1999	Study of prevalence, not screening.
Soergel 2006	Less than 80% follow-up.
Sokol 1998	Observation of Down's prevalence stratified by age.

(*Continued*)

Sonek 2003	Editorial.
Sonek 2007	Second trimester ultrasound.
Sood 2010	No diagnostic data.
Sooklim 2010	Second trimester ultrasound.
Spencer 1985	Fewer than 80% USS dated.
Spencer 1991a	Likely fewer than 80% USS dated.
Spencer 1991b	Unable to extract useful data.
Spencer 1992	Unable to extract useful data.
Spencer 1993a	Fewer than 80% USS dated.
Spencer 1993b	No Down's pregnancies in study population.
Spencer 1993c	Unable to extract useful data.
Spencer 1993d	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1993e	Unable to extract useful data.
Spencer 1995	No Down's pregnancies in population.
Spencer 1996	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1997	Statistical modelling, aneuploid pregnancies only in study population
Spencer 1998a	No Down's pregnancies in population.
Spencer 1998b	Unable to extract useful data.
Spencer 1999b	Review.
Spencer 1999c	Statistical methods paper.
Spencer 2000a	Examination of median shifts rather than an evaluation of screening
Spencer 2000b	No Down's syndrome pregnancies in population.
Spencer 2000c	No Down's syndrome pregnancies in population.
Spencer 2000d	No Down's cases.

(*Continued*)

Spencer 2000e	Male versus female fetuses.
Spencer 2000f	No Down's cases in population.
Spencer 2000g	No Down's pregnancies in population.
Spencer 2000h	No Down's pregnancies in population.
Spencer 2000i	Comparison of fetal sex.
Spencer 2001a	No Down's syndrome pregnancies in population.
Spencer 2001b	Unable to extract useful data.
Spencer 2001c	Unable to extract useful data.
Spencer 2001d	Unable to extract useful data.
Spencer 2001e	No Down's syndrome pregnancies in population.
Spencer 2002b	No Down's pregnancies.
Spencer 2002c	Risk validation study.
Spencer 2002d	No Down's syndrome pregnancies in population.
Spencer 2002e	Demonstration of median changes with time, rather than evaluation of screening
Spencer 2003a	No Down's pregnancies in population.
Spencer 2003b	No Down's pregnancies in population.
Spencer 2003c	Calculation of weight correction factor.
Spencer 2003d	Fewer than 5 Down's syndrome pregnancies.
Spencer 2004	Calculation of smoking correction factor.
Spencer 2005a	No Down's pregnancies.
Spencer 2005b	No Down's pregnancies.
Spencer 2005c	Comparison of 2 different assays - not actual screening evaluation
Spencer 2008	Unable to extract appropriate data for unaffected pregnancies

(*Continued*)

Spong 1999	Comparison of male and female fetuses.
Staboulidou 2009	No diagnostic data.
Stevens 1998	Literature review.
Stoll 1992	Review article.
Stressig 2011	Second trimester ultrasound.
Su 2002	Unable to extract useful data.
Suchet 1995	Review article.
Suchy 1990	Unable to ascertain method of confirmation of gestational age
Summers 2003a	Only 55% gestational ages estimated by USS.
Summers 2003b	No Down's syndrome pregnancies in study population.
Suntharasaj 2005	Examination of inter-observer variation in NT scanning.
Susman 2010	No diagnostic data.
Sutton 2004	Unable to extract useful data.
Suzuki 1998	Unable to extract useful data.
Tabor 1987	Gestational age not confirmed by USS.
Tanski 1999	Information on screen positive pregnancies only.
Thilaganathan 1998	No Down's syndrome pregnancies in study population.
Thilaganathan 1999	Editorial.
Tislaric 2002	No Down's syndrome pregnancies in population.
Torok 1997	Unable to extract useful data.
Torring 2009	Not possible to obtain full diagnostic data.
Trninic-Pjevic 2007	Unable to obtain translation.
Tsai 2001	Less than 5 Down's syndrome pregnancies in study population.
Valerio 1996	Fewer than 80% pregnancies had gestational age estimated by USS

(*Continued*)

Van Blerk 1992	Unable to extract useful data.
Van Dyke 2007	Not possible to obtain full diagnostic data.
Van Heesch, 2006	No Down's syndrome pregnancies in study population. Software comparison study
Van Lith 1991	Unable to extract useful data.
Van Lith 1993	Unable to extract useful data.
Van Lith 1994	Unable to extract useful data.
Veress 1986	Unable to extract useful data.
Veress 1988	Unable to extract useful data.
Vergani 2008	Second trimester ultrasound.
Vintzileos 2003	Second trimester USS.
Wald 1988a	Less than 80% had gestational age confirmed by ultrasound.
Wald 1988b	Gestational age not confirmed by USS.
Wald 1991	No Down's pregnancies in study.
Wald 1992a	Less than 80% had gestational age confirmed by ultrasound.
Wald 1992b	No Down's pregnancies in study.
Wald 1992c	No Down's pregnancies in study.
Wald 1993	No USS dating.
Wald 1994	No Down's syndrome pregnancies in population.
Wald 1994a	Review article.
Wald 1996a	No Down's pregnancies.
Wald 1996b	Dated by LMP.
Wald 1996c	No Down's syndrome pregnancies in population.
Wald 1996d	Gestational age greater than 24 weeks.
Wald 1997	Data modelled on 3 separate populations of women.

(*Continued*)

Wald 1998	Unable to extract useful data.
Wald 1999a	Unable to extract useful data.
Wald 1999b	Gestational age not confirmed by USS.
Wald 1999c	No Down's syndrome pregnancies.
Wald 1999d	Modelled on several studies, some of which have no USS dating
Wald 2003b	No cases.
Wald 2003c	Less than 80% had gestational age confirmed by USS.
Wald 2006	Modelled on SURRUS data.
Wallace 1994	Unable to extract useful data.
Wallace 1997	No Down's syndrome pregnancies in study population.
Wang 2010	Second trimester ultrasound.
Ward 2005	Review article.
Watt 1996a	No Down's syndrome pregnancies in study population.
Watt 1996b	No Down's syndrome pregnancies in study population.
Wax 2007	No diagnostic data.
Weinans 2001	Unable to extract useful data.
Weinans 2004	Study of women's views on screening.
Weisz 2007	Cohort split into people having different tests and non-representative samples of women assessed for each test
Welborn 1994	Abnormal results only (cystic hygroma).
Wenstrom 1993	Less than 80% of pregnancies had gestational age confirmed by USS
Wenstrom 1995a	Adjustment factors.
Wenstrom 1995b	Less than 80% of pregnancies had gestational age confirmed by USS
Wetta 2011	No diagnostic data.
Whitlow 1998a	Unable to extract useful data.

(*Continued*)

Whitlow 1998b	Unable to extract useful data.
Whitlow 1999	Unable to extract useful data.
Williamson 1994	Likely fewer than 80% USS dated.
Wilson 2000	Review.
Wojdemann 2001	No Down's syndrome pregnancies in study population.
Wong 2003	Less than 5 Down's syndrome pregnancies in population.
Wright 2006	Mathematical model.
Wright 2007	Simulation study, no new data.
Xie 2010	Only cases of false negatives and true negatives included.
Yagel 1998	Second trimester USS.
Yamamoto 2001a	Unable to extract useful data.
Yamamoto 2001b	Method of determination of gestational age unclear.
Yamamoto 2001c	Unable to extract useful data.
Yaron 2001	Male versus female fetuses.
Ye 1995	Unable to obtain translation.
Yoshida 2000	Fewer than 80% pregnancies had gestational age estimated by USS
Zalel 2008	No diagnostic data.
Zeitune 1991	Only aneuploid pregnancies included in study.
Zelop 2005	No Down's cases in population.
Zhang 2011	No diagnostic data.
Zhao 1998	Unable to obtain translation.
Zhong 2011	Second trimester ultrasound.
Zoppi 2003	Inappropriate study design.

CME: continuing medical education

CVS: chorionic villus sampling
DTA: diagnostic test accuracy
FISH technique: fluorescence in situhybridization
LMP: last menstrual period
NT**:** nuchal translucency
USS: ultrasound scan

DATA

Presented below are all the data for all of the tests entered into the review.

Tests. Data tables by test

Test	No. of studies	No. of participants
1 1T PAPP-A, 5% FPR	4	2837
2 1T PAPP-A, $\leq 5^{th}$ percentile	1	22280
3 1T PAPP-A, mixed cut-points	6	25510
4 1T free ßhCG, 5% FPR	4	4280
5 1T total hCG, 5FPR	2	2482
6 1T AFP, 5% FPR	2	2248
10 1T AFP, mixed cut-points	3	2724
11 1T Inhibin, 5FPR	3	2098
12 1T ADAM 12, 5FPR	1	579
13 1T SP1, 5% FPR	3	1080
17 ba˙hcg˙ratio, 0.25MoM	1	476
18 1T uE3, 5% FPR	1	1110
19 1T PlGF, 5FPR	1	699
20 1T PAPP-A and 1T free ßhCG, 5% FPR	2	795
21 1T PAPP-A and 1T free ßhCG, mixed cut-points	2	795
22 1T PAPP-A and 1T AFP, 5% FPR	1	96
23 1T PAPP-A and 1T ITA, 3% FPR	1	344
24 1T PAPP-A and 1T ITA, 5% FPR	1	344
25 1T free ßhCG and 1T Inhibin, 5% FPR	1	876
26 1T free ßhCG and 1T AFP, 5% FPR	1	1138
27 1T PAPP-A and 1T ITA, 10% FPR	1	344
28 1T PAPP-A, 1T free ßhCG and 1T ITA, 5% FPR	1	344
29 1T PAPP-A, 1T free ßhCG and 1T ITA,3% FPR	1	344
30 1T PAPP-A, 1T free ßhCG and 1T ITA, 10% FPR	1	344
31 1T total hCG, 1T free αhCG and 1T progesterone, 0.34 MoM	1	129
32 Age, 1T Inhibin, risk 1:100	1	40
33 Age, 1T Inhibin, risk 1:250	1	40
34 Age, 1T Inhibin, risk 1:400	1	40

35 Age, 1T Inhibin, 5FPR	1	1110
36 Age, 1T Inhibin, mixed cut-points	2	1150
37 Age, 1T PAPP-A, 5FPR	5	3491
38 Age, 1T PAPP-A, mixed cut-points	6	13742
39 Age, 1T free ßhCG, 5FPR	7	5893
40 Age, 1T free ßhCG, risk 1:384	1	512
41 Age, 1T free ßhCG, mixed cut-points	9	16656
42 Age, 1T total hCG,risk 1:384	1	512
43 Age, 1T total hCG, mixed cut-points	2	1622
44 Age, 1T AFP, 5FPR	2	1397
45 Age, 1T AFP, risk1:384	1	512
46 Age, 1T AFP,mixed cut-points	3	1909
47 Age, 1T uE3, risk 1:384	1	512
48 Age, 1T uE3, mixed cut-points	2	799
49 Age, 1T free αhCG, risk 1:384	1	512
50 Age, 1T SP1, 5FPR	2	804
51 Age, 1T ProMBP, risk 1:250	1	181
52 Age, 1T ITA, 5FPR	1	278
53 Age, 1T ADAM 12, risk 1:400	2	703
54 Age, 1T PAPP-A and 1T free ßhCG, risk 1:250	11	60484
55 Age, 1T PAPP-A and 1T free ßhCG, 5FPR	17	49827
56 Age, 1T PAPP-A and 1T free ßhCG, mixed cut-points	31	158878
57 Age, 1T PAPP-A and 1T free ßhCG, mixed cut-points without 5FPR	20	138731
58 Age, 1T total hCG and 1T PAPP-A, 5FPR	2	4327
59 Age, 1T PAPP-A and 1T Inhibin, risk 1:100	1	41
60 Age, 1T PAPP-A and 1T Inhibin, risk 1:250	1	40
61 Age, 1T PAPP-A and 1T Inhibin, risk 1:400	1	40
62 Age, 1T PAPP-A and 1T Inhibin, 5FPR	1	1110
63 Age, 1T PAPP-A and 1T Inhibin, mixed cut-points	2	1150
64 Age, 1T PAPP-A and 1T ITA, 5FPR	2	622
65 Age, 1T PAPP-A and 1T AFP, 5FPR	2	2705
66 Age, 1T free ßhCG and 1T Inhibin, risk 1:100	1	40

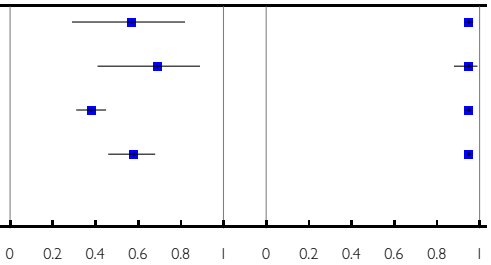
116 Age, 1T GHBP, 1T free ßhCG, risk 1:250	1	335
117 Age, 1T GHBP, 1T PGH, risk 1:250	1	335
118 Age, 1T GHBP, 1T PAPP-A, 1T free ßhCG , risk 1:250	1	335
119 Age, 1T GHBP, 1T PGH, 1T PAPP-A, 1T free ßhCG , risk 1:250	1	335
120 Age, 1T ADAM 12, risk 1:250	1	531
121 Age, 1T ADAM 12, 1T PAPP-A, 1T free ßhCG, risk 1:250	3	1501
122 Age, PlGF, 1T PAPP-A, 1T free ßhCG, 5FPR	2	1144
123 Age, 1T PAPP-A and 1T free ßhCG, risk 1:300	4	41172
124 Age, 1T PAPP-A, 1T Hyperglycosylated hCG, 5FPR	1	10775
128 Age, ADAM 12, 1T PAPP-A, 5FPR	1	691
129 Age, ADAM 12, 1T PAPP-A, 1T free ßhCG, 5FPR	2	1222
130 Age, 1T PlGF, 5FPR	1	699
131 1T PlGF, 1T PAPP-A, 1T free ßhCG, 5FPR	1	699
132 Age, 1T ADAM 12, 1T PAPP-A, 1T free ßhCG, mixed cut-points	3	1501
133 Age, 1T PAPP-A, 1T free ßhCG and 1T Inhibin, risk 1:250	1	40
134 Age, 1T PAPP-A, 1T free ßhCG, and 1T Inhibin, mixed cut-points	2	1150

Test 1. 1T PAPP-A, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 1 1T PAPP-A, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brambati 1993	8	23	6	438	0.57 [0.29, 0.82]	0.95 [0.93, 0.97]
Casals 1996	11	4	5	76	0.69 [0.41, 0.89]	0.95 [0.88, 0.99]
Spencer 1999a	80	47	130	899	0.38 [0.31, 0.45]	0.95 [0.93, 0.96]
Wald 2003a	49	51	36	974	0.58 [0.46, 0.68]	0.95 [0.94, 0.96]

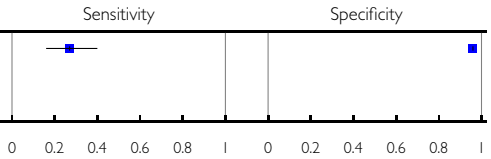


Test 2. 1T PAPP-A, $\leq$5th percentile.

Review: First trimester serum tests for Down's syndrome screening

Test: 2 1T PAPP-A, $\leq 5^{th}$ percentile

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brameld 2008	16	922	44	21298	0.27 [0.16, 0.40]	0.96 [0.96, 0.96]

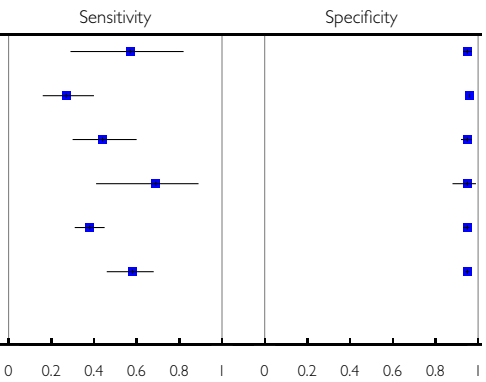


Test 3. 1T PAPP-A, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 3 1T PAPP-A, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brambati 1993	8	23	6	438	0.57 [0.29, 0.82]	0.95 [0.93, 0.97]
Brameld 2008	16	922	44	21298	0.27 [0.16, 0.40]	0.96 [0.96, 0.96]
Brizot 1994	20	17	25	331	0.44 [0.30, 0.60]	0.95 [0.92, 0.97]
Casals 1996	11	4	5	76	0.69 [0.41, 0.89]	0.95 [0.88, 0.99]
Spencer 1999a	80	47	130	899	0.38 [0.31, 0.45]	0.95 [0.93, 0.96]
Wald 2003a	49	51	36	974	0.58 [0.46, 0.68]	0.95 [0.94, 0.96]

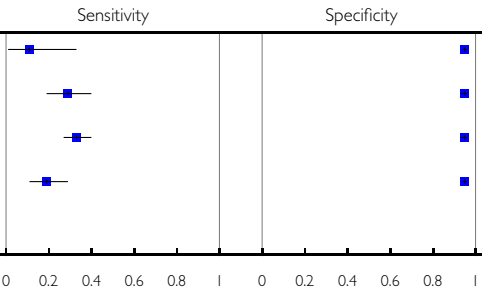


Test 4. 1T free ßhCG, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 4 1T free hCG, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Casals 1996	2	56	17	1063	0.11 [0.01, 0.33]	0.95 [0.94, 0.96]
Noble 1997	22	40	54	760	0.29 [0.19, 0.40]	0.95 [0.93, 0.96]
Spencer 1999a	69	47	141	899	0.33 [0.27, 0.40]	0.95 [0.93, 0.96]
Wald 2003a	16	51	69	974	0.19 [0.11, 0.29]	0.95 [0.94, 0.96]

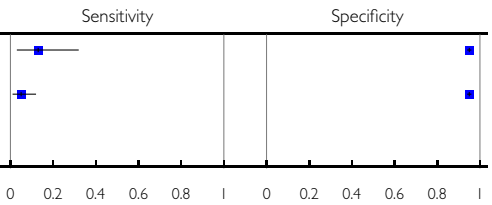


Test 5. 1T total hCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 5 1T total hCG, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Van Lith 1992	3	67	21	1281	0.13 [0.03, 0.32]	0.95 [0.94, 0.96]
Wald 2003a	4	51	81	974	0.05 [0.01, 0.12]	0.95 [0.94, 0.96]

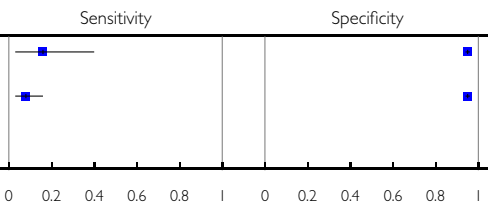


Test 6. 1T AFP, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 6 1T AFP, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Casals 1996	3	56	16	1063	0.16 [0.03, 0.40]	0.95 [0.94, 0.96]
Wald 2003a	7	51	78	974	0.08 [0.03, 0.16]	0.95 [0.94, 0.96]

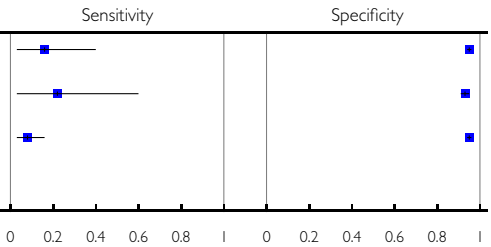


Test 10. 1T AFP, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 10 1T AFP, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Casals 1996	3	56	16	1063	0.16 [0.03, 0.40]	0.95 [0.94, 0.96]
Nebiolo 1990	2	31	7	436	0.22 [0.03, 0.60]	0.93 [0.91, 0.95]
Wald 2003a	7	51	78	974	0.08 [0.03, 0.16]	0.95 [0.94, 0.96]

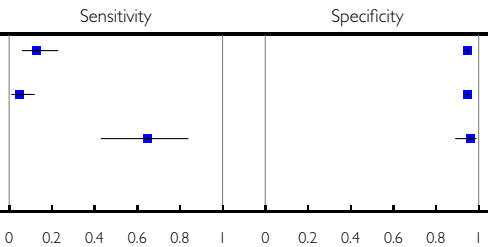


Test 11. 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 11 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Noble 1997	10	40	66	760	0.13 [0.06, 0.23]	0.95 [0.93, 0.96]
Wald 2003a	4	51	81	974	0.05 [0.01, 0.12]	0.95 [0.94, 0.96]
Wallace 1995	15	4	8	85	0.65 [0.43, 0.84]	0.96 [0.89, 0.99]

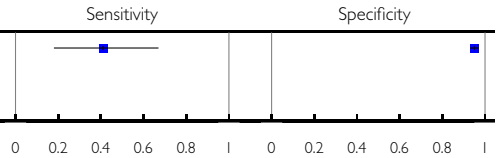


Test 12. 1T ADAM 12, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 12 1T ADAM 12, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Baviera 2010	7	28	10	534	0.41 [0.18, 0.67]	0.95 [0.93, 0.97]

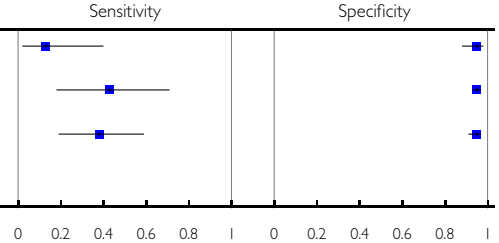


Test 13. 1T SP1, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 13 1T SP1, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Kornman 1998	2	5	13	92	0.13 [0.02, 0.40]	0.95 [0.88, 0.98]
Macintosh 1993	6	34	8	644	0.43 [0.18, 0.71]	0.95 [0.93, 0.97]
Qin 1997	9	13	15	239	0.38 [0.19, 0.59]	0.95 [0.91, 0.97]

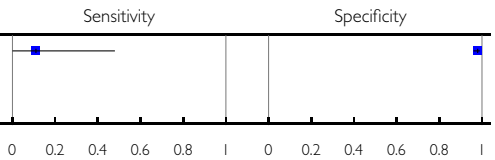


Test 17. ba˙hcg˙ratio, 0.25MoM.

Review: First trimester serum tests for Down's syndrome screening

Test: 17 ba˙hcg˙ratio, 0.25MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Nebiolo 1990	1	10	8	457	0.11 [0.00, 0.48]	0.98 [0.96, 0.99]

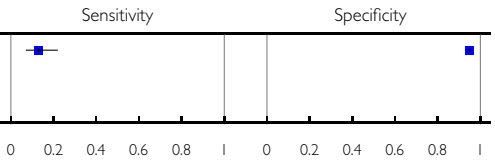


Test 18. 1T uE3, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 18 1T uE3, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	11	51	74	974	0.13 [0.07, 0.22]	0.95 [0.94, 0.96]

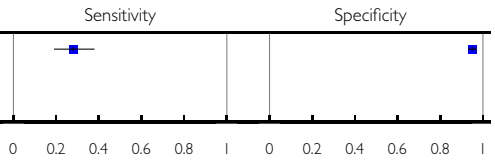


Test 19. 1T PlGF, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 19 1T PlGF, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Zaragoza 2009	25	30	65	579	0.28 [0.19, 0.38]	0.95 [0.93, 0.97]

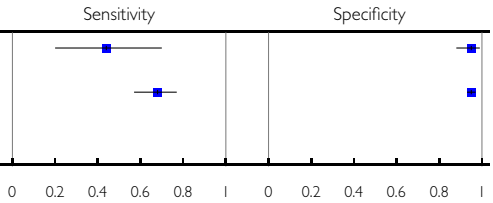


Test 20. 1T PAPP-A and 1T free ßhCG, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 20 1T PAPP-A and 1T free hCG, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Casals 1996	7	4	9	76	0.44 [0.20, 0.70]	0.95 [0.88, 0.99]
Zaragoza 2009	61	30	29	579	0.68 [0.57, 0.77]	0.95 [0.93, 0.97]

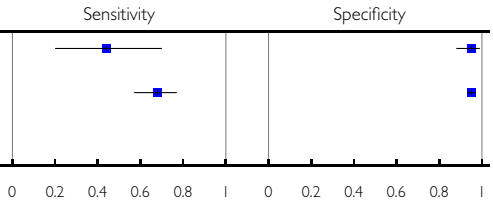


Test 21. 1T PAPP-A and 1T free ßhCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 21 1T PAPP-A and 1T free hCG, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Casals 1996	7	4	9	76	0.44 [0.20, 0.70]	0.95 [0.88, 0.99]
Zaragoza 2009	61	30	29	579	0.68 [0.57, 0.77]	0.95 [0.93, 0.97]

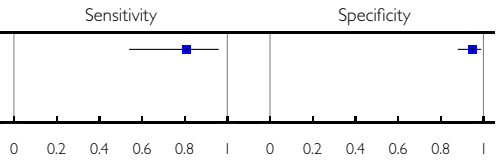


Test 22. 1T PAPP-A and 1T AFP, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 22 1T PAPP-A and 1T AFP, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Casals 1996	13	4	3	76	0.81 [0.54, 0.96]	0.95 [0.88, 0.99]

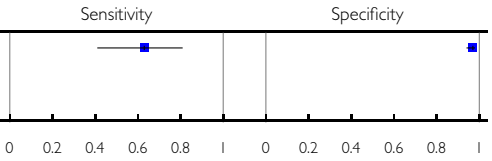


Test 23. 1T PAPP-A and 1T ITA, 3% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 23 1T PAPP-A and 1T ITA, 3% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Weinans 2005	15	10	9	310	0.63 [0.41, 0.81]	0.97 [0.94, 0.98]

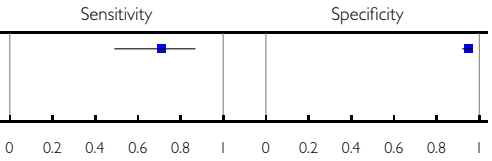


Test 24. 1T PAPP-A and 1T ITA, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 24 1T PAPP-A and 1T ITA, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Weinans 2005	17	16	7	304	0.71 [0.49, 0.87]	0.95 [0.92, 0.97]

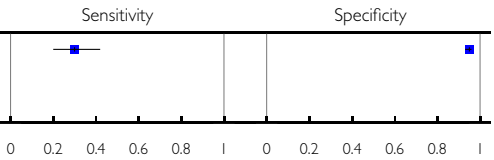


Test 25. 1T free ßhCG and 1T Inhibin, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 25 1T free hCG and 1T Inhibin, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Noble 1997	23	40	53	760	0.30 [0.20, 0.42]	0.95 [0.93, 0.96]

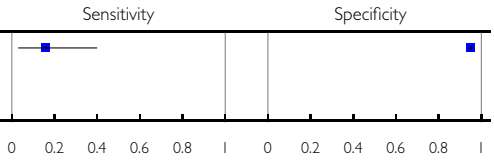


Test 26. 1T free ßhCG and 1T AFP, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 26 1T free hCG and 1T AFP, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Casals 1996	3	56	16	1063	0.16 [0.03, 0.40]	0.95 [0.94, 0.96]

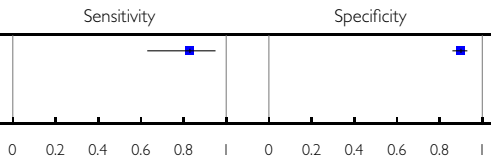


Test 27. 1T PAPP-A and 1T ITA, 10% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 27 1T PAPP-A and 1T ITA, 10% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Weinans 2005	20	32	4	288	0.83 [0.63, 0.95]	0.90 [0.86, 0.93]



Test 28. 1T PAPP-A, 1T free ßhCG and 1T ITA, 5% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 28 1T PAPP-A, 1T free hCG and 1T ITA, 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Weinans 2005	18	16	6	304	0.75 [0.53, 0.90]	0.95 [0.92, 0.97]		

Test 29. 1T PAPP-A, 1T free ßhCG and 1T ITA,3% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 29 1T PAPP-A, 1T free hCG and 1T ITA,3% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Weinans 2005	14	10	10	310	0.58 [0.37, 0.78]	0.97 [0.94, 0.98]		

Test 30. 1T PAPP-A, 1T free ßhCG and 1T ITA, 10% FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 30 1T PAPP-A, 1T free hCG and 1T ITA, 10% FPR

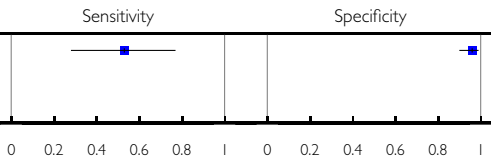
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Weinans 2005	19	32	5	288	0.79 [0.58, 0.93]	0.90 [0.86, 0.93]		

Test 31. 1T total hCG, 1T free αhCG and 1T progesterone, 0.34 MoM.

Review: First trimester serum tests for Down's syndrome screening

Test: 31 1T total hCG, 1T free α hCG and 1T progesterone, 0.34 MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Kratzer 1991	9	5	8	107	0.53 [0.28, 0.77]	0.96 [0.90, 0.99]

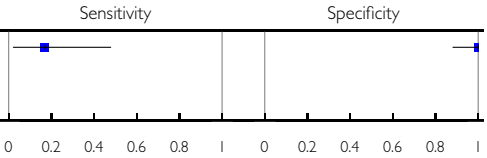


Test 32. Age, 1T Inhibin, risk 1:100.

Review: First trimester serum tests for Down's syndrome screening

Test: 32 Age, 1T Inhibin, risk 1:100

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	2	0	10	28	0.17 [0.02, 0.48]	1.00 [0.88, 1.00]

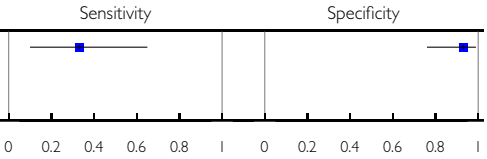


Test 33. Age, 1T Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 33 Age, 1T Inhibin, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	4	2	8	26	0.33 [0.10, 0.65]	0.93 [0.76, 0.99]

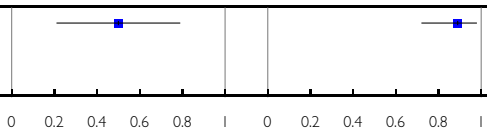


Test 34. Age, 1T Inhibin, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

Test: 34 Age, 1T Inhibin, risk 1:400

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	6	3	6	25	0.50 [0.21, 0.79]	0.89 [0.72, 0.98]		

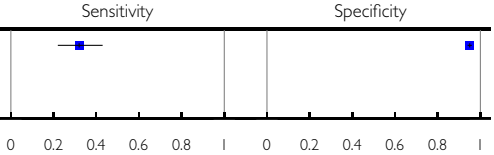


Test 35. Age, 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 35 Age, 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003a	27	51	58	974	0.32 [0.22, 0.43]	0.95 [0.94, 0.96]		

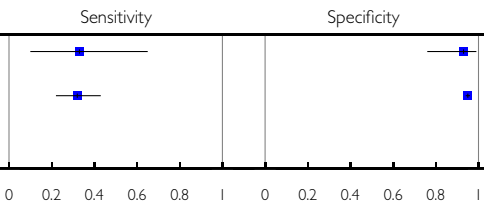


Test 36. Age, 1T Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 36 Age, 1T Inhibin, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	4	2	8	26	0.33 [0.10, 0.65]	0.93 [0.76, 0.99]
Wald 2003a	27	51	58	974	0.32 [0.22, 0.43]	0.95 [0.94, 0.96]

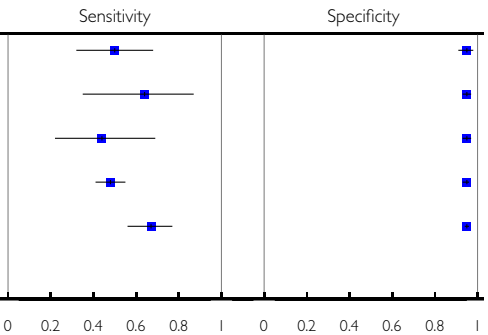


Test 37. Age, 1T PAPP-A, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 37 Age, 1T PAPP-A, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1998	16	10	16	190	0.50 [0.32, 0.68]	0.95 [0.91, 0.98]
Brambati 1993	9	23	5	438	0.64 [0.35, 0.87]	0.95 [0.93, 0.97]
Forest 1997	8	25	10	475	0.44 [0.22, 0.69]	0.95 [0.93, 0.97]
Spencer 1999a	101	47	109	899	0.48 [0.41, 0.55]	0.95 [0.93, 0.96]
Wald 2003a	57	51	28	974	0.67 [0.56, 0.77]	0.95 [0.94, 0.96]

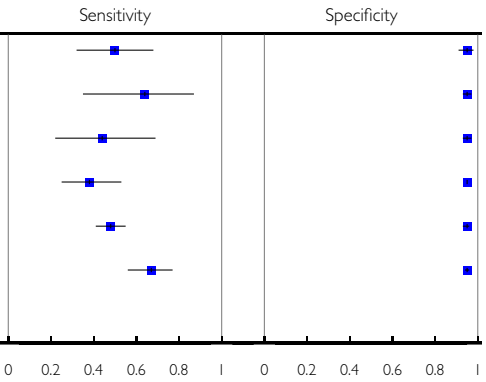


Test 38. Age, 1T PAPP-A, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 38 Age, 1T PAPP-A, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1998	16	10	16	190	0.50 [0.32, 0.68]	0.95 [0.91, 0.98]
Brambati 1993	9	23	5	438	0.64 [0.35, 0.87]	0.95 [0.93, 0.97]
Forest 1997	8	25	10	475	0.44 [0.22, 0.69]	0.95 [0.93, 0.97]
Krantz 2000	19	510	31	9691	0.38 [0.25, 0.53]	0.95 [0.95, 0.95]
Spencer 1999a	101	47	109	899	0.48 [0.41, 0.55]	0.95 [0.93, 0.96]
Wald 2003a	57	51	28	974	0.67 [0.56, 0.77]	0.95 [0.94, 0.96]

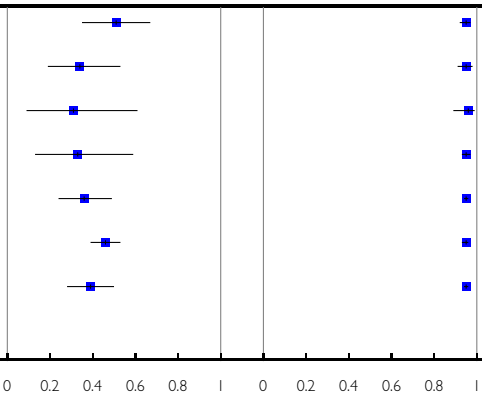


Test 39. Age, 1T free ßhCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 39 Age, 1T free hCG, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	21	12	20	234	0.51 [0.35, 0.67]	0.95 [0.92, 0.97]
Biagiotti 1998	11	10	21	190	0.34 [0.19, 0.53]	0.95 [0.91, 0.98]
Brambati 1994	4	4	9	85	0.31 [0.09, 0.61]	0.96 [0.89, 0.99]
Forest 1997	6	25	12	475	0.33 [0.13, 0.59]	0.95 [0.93, 0.97]
Noble 1995	22	121	39	2306	0.36 [0.24, 0.49]	0.95 [0.94, 0.96]
Spencer 1999a	97	47	113	899	0.46 [0.39, 0.53]	0.95 [0.93, 0.96]
Wald 2003a	33	51	52	974	0.39 [0.28, 0.50]	0.95 [0.94, 0.96]

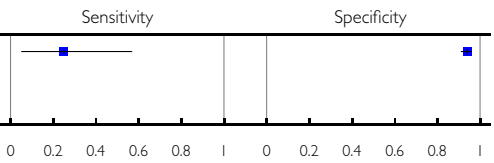


Test 40. Age, 1T free ßhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 40 Age, 1T free hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	3	32	9	468	0.25 [0.05, 0.57]	0.94 [0.91, 0.96]

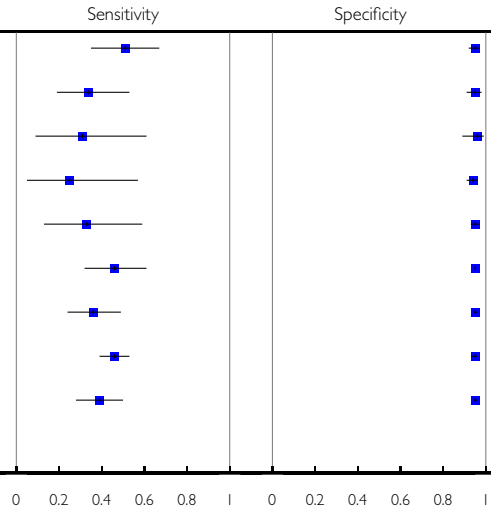


Test 41. Age, 1T free ßhCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 41 Age, 1T free hCG, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	21	12	20	234	0.51 [0.35, 0.67]	0.95 [0.92, 0.97]
Biagiotti 1998	11	10	21	190	0.34 [0.19, 0.53]	0.95 [0.91, 0.98]
Brambati 1994	4	4	9	85	0.31 [0.09, 0.61]	0.96 [0.89, 0.99]
Forest 1995	3	32	9	468	0.25 [0.05, 0.57]	0.94 [0.91, 0.96]
Forest 1997	6	25	12	475	0.33 [0.13, 0.59]	0.95 [0.93, 0.97]
Krantz 2000	23	510	27	9691	0.46 [0.32, 0.61]	0.95 [0.95, 0.95]
Noble 1995	22	121	39	2306	0.36 [0.24, 0.49]	0.95 [0.94, 0.96]
Spencer 1999a	97	47	113	899	0.46 [0.39, 0.53]	0.95 [0.93, 0.96]
Wald 2003a	33	51	52	974	0.39 [0.28, 0.50]	0.95 [0.94, 0.96]

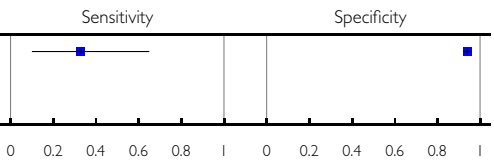


Test 42. Age, 1T total hCG,risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 42 Age, 1T total hCG,risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	4	30	8	470	0.33 [0.10, 0.65]	0.94 [0.92, 0.96]

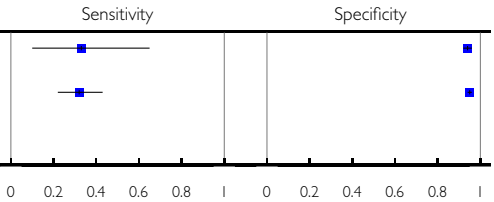


Test 43. Age, 1T total hCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 43 Age, 1T total hCG, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	4	30	8	470	0.33 [0.10, 0.65]	0.94 [0.92, 0.96]
Wald 2003a	27	51	58	974	0.32 [0.22, 0.43]	0.95 [0.94, 0.96]

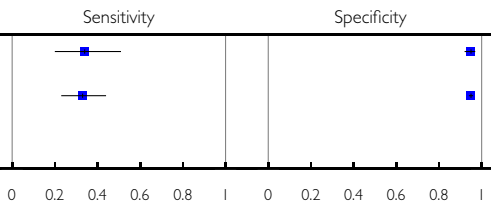


Test 44. Age, 1T AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 44 Age, 1T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	14	12	27	234	0.34 [0.20, 0.51]	0.95 [0.92, 0.97]
Wald 2003a	28	51	57	974	0.33 [0.23, 0.44]	0.95 [0.94, 0.96]

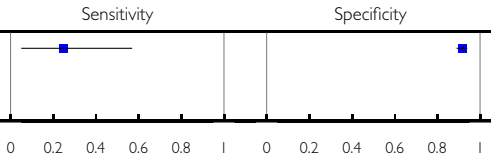


Test 45. Age, 1T AFP, risk1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 45 Age, 1T AFP, risk1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	3	39	9	461	0.25 [0.05, 0.57]	0.92 [0.89, 0.94]

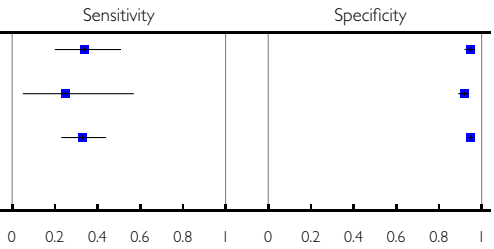


Test 46. Age, 1T AFP,mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 46 Age, 1T AFP,mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	14	12	27	234	0.34 [0.20, 0.51]	0.95 [0.92, 0.97]
Forest 1995	3	39	9	461	0.25 [0.05, 0.57]	0.92 [0.89, 0.94]
Wald 2003a	28	51	57	974	0.33 [0.23, 0.44]	0.95 [0.94, 0.96]

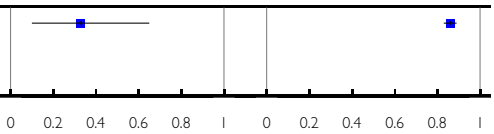


Test 47. Age, 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 47 Age, 1T uE3, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	4	70	8	430	0.33 [0.10, 0.65]	0.86 [0.83, 0.89]



Test 48. Age, 1T uE3, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 48 Age, 1T uE3, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Biagiotti 1995	17	12	24	234	0.41 [0.26, 0.58]	0.95 [0.92, 0.97]		
Forest 1995	4	70	8	430	0.33 [0.10, 0.65]	0.86 [0.83, 0.89]		

Test 49. Age, 1T free αhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 49 Age, 1T free α hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	3	56	9	444	0.25 [0.05, 0.57]	0.89 [0.86, 0.91]		

Test 50. Age, 1T SP1, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 50 Age, 1T SP1, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Kornman 1998	4	5	11	92	0.27 [0.08, 0.55]	0.95 [0.88, 0.98]		
Macintosh 1993	7	34	7	644	0.50 [0.23, 0.77]	0.95 [0.93, 0.97]		

Test 51. Age, 1T ProMBP, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 51 Age, 1T ProMBP, risk 1:250

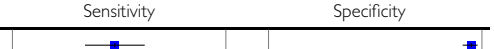
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 1999	9	9	16	147	0.36 [0.18, 0.57]	0.94 [0.89, 0.97]		

Test 52. Age, 1T ITA, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 52 Age, 1T ITA, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Spencer 2002a	26	11	28	213	0.48 [0.34, 0.62]	0.95 [0.91, 0.98]		

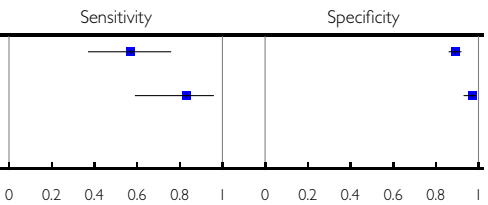


Test 53. Age, 1T ADAM 12, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

Test: 53 Age, 1T ADAM 12, risk 1:400

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2010	16	55	12	448	0.57 [0.37, 0.76]	0.89 [0.86, 0.92]
Laigaard 2003	15	5	3	149	0.83 [0.59, 0.96]	0.97 [0.93, 0.99]

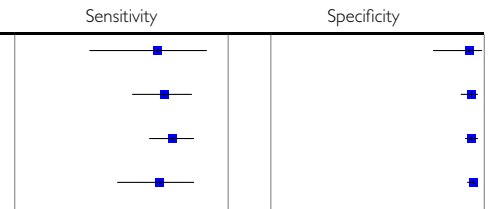


Test 54. Age, 1T PAPP-A and 1T free ßhCG, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 54 Age, 1T PAPP-A and 1T free hCG, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	8	2	4	26	0.67 [0.35, 0.90]	0.93 [0.76, 0.99]
Christiansen 2007a	33	8	14	128	0.70 [0.55, 0.83]	0.94 [0.89, 0.97]
Christiansen 2009	55	15	19	246	0.74 [0.63, 0.84]	0.94 [0.91, 0.97]
Christiansen 2010	19	27	9	476	0.68 [0.48, 0.84]	0.95 [0.92, 0.96]
Crossley 2002a	23	848	19	16105	0.55 [0.39, 0.70]	0.95 [0.95, 0.95]
Kagan 2009	96	2079	26	17535	0.79 [0.70, 0.86]	0.89 [0.89, 0.90]
Muller 2003a	18	437	8	5020	0.69 [0.48, 0.86]	0.92 [0.91, 0.93]
Niemimaa 2001a	6	243	2	2264	0.75 [0.35, 0.97]	0.90 [0.89, 0.91]
Torring 2010	40	61	6	584	0.87 [0.74, 0.95]	0.91 [0.88, 0.93]



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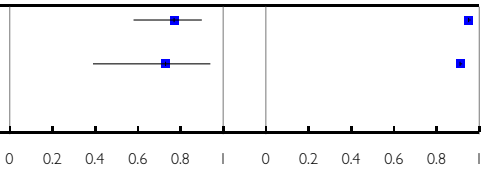
Study	TP	FP	FN	TN	Sensitivity	Specificity
Valinen 2007	23	360	7	7144	0.77 [0.58, 0.90]	0.95 [0.95, 0.96]
Wojdemann 2005	8	564	3	5866	0.73 [0.39, 0.94]	0.91 [0.91, 0.92]

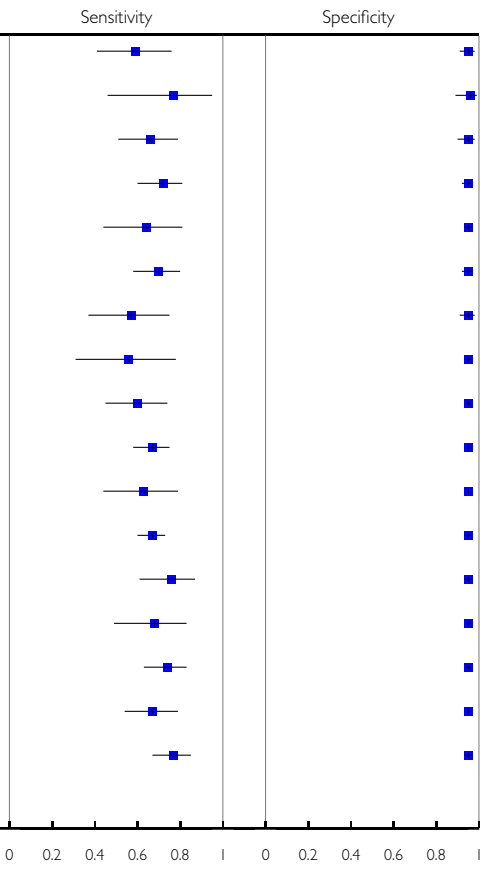


Test 55. Age, 1T PAPP-A and 1T free ßhCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 55 Age, 1T PAPP-A and 1T free hCG, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1998	19	10	13	190	0.59 [0.41, 0.76]	0.95 [0.91, 0.98]
Brambati 1994	10	4	3	85	0.77 [0.46, 0.95]	0.96 [0.89, 0.99]
Christiansen 2007a	31	7	16	129	0.66 [0.51, 0.79]	0.95 [0.90, 0.98]
Christiansen 2009	53	13	21	248	0.72 [0.60, 0.81]	0.95 [0.92, 0.97]
Christiansen 2010	18	25	10	478	0.64 [0.44, 0.81]	0.95 [0.93, 0.97]
Cowans 2010	49	19	21	356	0.70 [0.58, 0.80]	0.95 [0.92, 0.97]
De Graaf 1999a	17	9	13	168	0.57 [0.37, 0.75]	0.95 [0.91, 0.98]
Forest 1997	10	25	8	475	0.56 [0.31, 0.78]	0.95 [0.93, 0.97]
Haddow 1998	29	158	19	3011	0.60 [0.45, 0.74]	0.95 [0.94, 0.96]
Kagan 2009	82	981	40	18633	0.67 [0.58, 0.75]	0.95 [0.95, 0.95]
Sahota 2010	20	541	12	10281	0.63 [0.44, 0.79]	0.95 [0.95, 0.95]
Spencer 1999a	141	47	69	899	0.67 [0.60, 0.73]	0.95 [0.93, 0.96]
Torring 2010	35	32	11	613	0.76 [0.61, 0.87]	0.95 [0.93, 0.97]
Tsukerman 1999	21	78	10	1486	0.68 [0.49, 0.83]	0.95 [0.94, 0.96]
Wald 2003a	63	51	22	974	0.74 [0.63, 0.83]	0.95 [0.94, 0.96]
Wapner 2003	41	408	20	7747	0.67 [0.54, 0.79]	0.95 [0.95, 0.95]
Zaragoza 2009	69	30	21	579	0.77 [0.67, 0.85]	0.95 [0.93, 0.97]

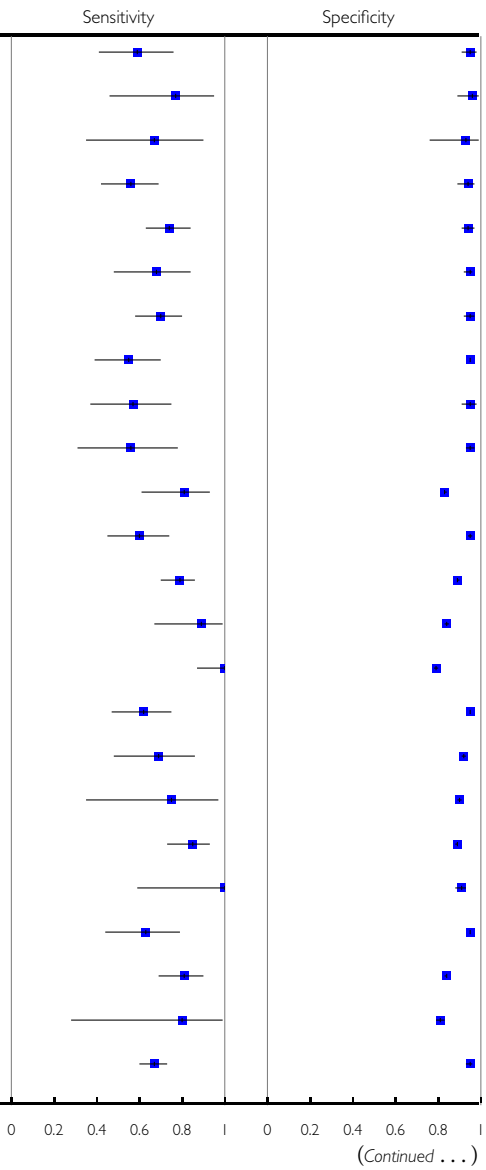


Test 56. Age, 1T PAPP-A and 1T free ßhCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 56 Age, 1T PAPP-A and 1T free hCG, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1998	19	10	13	190	0.59 [0.41, 0.76]	0.95 [0.91, 0.98]
Brambati 1994	10	4	3	85	0.77 [0.46, 0.95]	0.96 [0.89, 0.99]
Christiansen 2005	8	2	4	26	0.67 [0.35, 0.90]	0.93 [0.76, 0.99]
Christiansen 2007a	33	8	26	128	0.56 [0.42, 0.69]	0.94 [0.89, 0.97]
Christiansen 2009	55	15	19	246	0.74 [0.63, 0.84]	0.94 [0.91, 0.97]
Christiansen 2010	19	27	9	476	0.68 [0.48, 0.84]	0.95 [0.92, 0.96]
Cowans 2010	49	19	21	356	0.70 [0.58, 0.80]	0.95 [0.92, 0.97]
Crossley 2002a	23	848	19	16105	0.55 [0.39, 0.70]	0.95 [0.95, 0.95]
De Graaf 1999a	17	9	13	168	0.57 [0.37, 0.75]	0.95 [0.91, 0.98]
Forest 1997	10	25	8	475	0.56 [0.31, 0.78]	0.95 [0.93, 0.97]
Gyselaers 2005	21	2212	5	10969	0.81 [0.61, 0.93]	0.83 [0.83, 0.84]
Haddow 1998	29	158	19	3011	0.60 [0.45, 0.74]	0.95 [0.94, 0.96]
Kagan 2009	96	2079	26	17535	0.79 [0.70, 0.86]	0.89 [0.89, 0.90]
Kozlowski 2007 GC	17	1081	2	5806	0.89 [0.67, 0.99]	0.84 [0.83, 0.85]
Kozlowski 2007 PC	26	802	0	3034	1.00 [0.87, 1.00]	0.79 [0.78, 0.80]
Krantz 2000	31	510	19	9691	0.62 [0.47, 0.75]	0.95 [0.95, 0.95]
Muller 2003a	18	437	8	5020	0.69 [0.48, 0.86]	0.92 [0.91, 0.93]
Niemimaa 2001a	6	243	2	2264	0.75 [0.35, 0.97]	0.90 [0.89, 0.91]
O'Leary 2006	51	2560	9	19720	0.85 [0.73, 0.93]	0.89 [0.88, 0.89]
Orlandi 1997	7	68	0	669	1.00 [0.59, 1.00]	0.91 [0.88, 0.93]
Sahota 2010	20	541	12	10281	0.63 [0.44, 0.79]	0.95 [0.95, 0.95]
Schaelike 2009	48	1730	11	8879	0.81 [0.69, 0.90]	0.84 [0.83, 0.84]
Scott 2004	4	390	1	1658	0.80 [0.28, 0.99]	0.81 [0.79, 0.83]
Spencer 1999a	141	47	69	899	0.67 [0.60, 0.73]	0.95 [0.93, 0.96]



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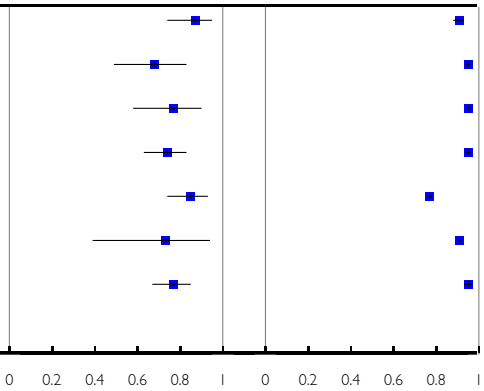
Study	TP	FP	FN	TN	Sensitivity	Specificity
Torring 2010	40	61	6	584	0.87 [0.74, 0.95]	0.91 [0.88, 0.93]
Tsukerman 1999	21	78	10	1486	0.68 [0.49, 0.83]	0.95 [0.94, 0.96]
Valinen 2007	23	360	7	7144	0.77 [0.58, 0.90]	0.95 [0.95, 0.96]
Wald 2003a	63	51	22	974	0.74 [0.63, 0.83]	0.95 [0.94, 0.96]
Wapner 2003	52	1892	9	6263	0.85 [0.74, 0.93]	0.77 [0.76, 0.78]
Wojdemann 2005	8	564	3	5866	0.73 [0.39, 0.94]	0.91 [0.91, 0.92]
Zaragoza 2009	69	30	21	579	0.77 [0.67, 0.85]	0.95 [0.93, 0.97]

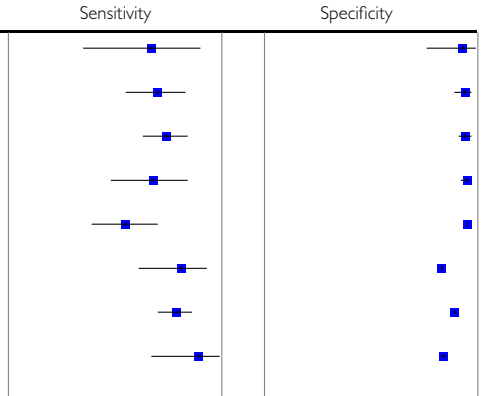


Test 57. Age, 1T PAPP-A and 1T free ßhCG, mixed cut-points without 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 57 Age, 1T PAPP-A and 1T free hCG, mixed cut-points without 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	8	2	4	26	0.67 [0.35, 0.90]	0.93 [0.76, 0.99]
Christiansen 2007a	33	8	14	128	0.70 [0.55, 0.83]	0.94 [0.89, 0.97]
Christiansen 2009	55	15	19	246	0.74 [0.63, 0.84]	0.94 [0.91, 0.97]
Christiansen 2010	19	27	9	476	0.68 [0.48, 0.84]	0.95 [0.92, 0.96]
Crossley 2002a	23	848	19	16105	0.55 [0.39, 0.70]	0.95 [0.95, 0.95]
Gyselaers 2005	21	2212	5	10969	0.81 [0.61, 0.93]	0.83 [0.83, 0.84]
Kagan 2009	96	2079	26	17535	0.79 [0.70, 0.86]	0.89 [0.89, 0.90]
Kozlowski 2007 GC	17	1081	2	5806	0.89 [0.67, 0.99]	0.84 [0.83, 0.85]
Kozlowski 2007 PC	26	802	0	3034	1.00 [0.87, 1.00]	0.79 [0.78, 0.80]
Krantz 2000	31	510	19	9691	0.62 [0.47, 0.75]	0.95 [0.95, 0.95]
Muller 2003a	18	437	8	5020	0.69 [0.48, 0.86]	0.92 [0.91, 0.93]
Niemimaa 2001a	6	243	2	2264	0.75 [0.35, 0.97]	0.90 [0.89, 0.91]
O'Leary 2006	51	2560	9	19720	0.85 [0.73, 0.93]	0.89 [0.88, 0.89]



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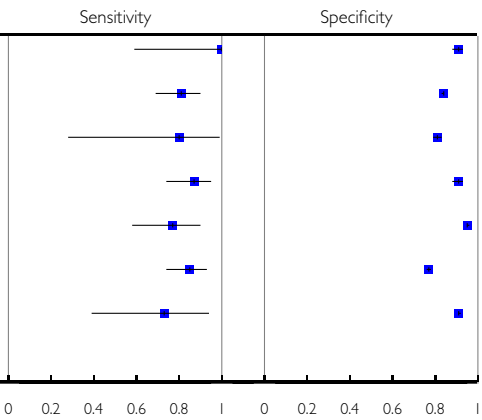
Study	TP	FP	FN	TN	Sensitivity	Specificity
Orlandi 1997	7	68	0	669	1.00 [0.59, 1.00]	0.91 [0.88, 0.93]
Schaelike 2009	48	1730	11	8879	0.81 [0.69, 0.90]	0.84 [0.83, 0.84]
Scott 2004	4	390	1	1658	0.80 [0.28, 0.99]	0.81 [0.79, 0.83]
Torring 2010	40	61	6	584	0.87 [0.74, 0.95]	0.91 [0.88, 0.93]
Valinen 2007	23	360	7	7144	0.77 [0.58, 0.90]	0.95 [0.95, 0.96]
Wapner 2003	52	1892	9	6263	0.85 [0.74, 0.93]	0.77 [0.76, 0.78]
Wojdemann 2005	8	564	3	5866	0.73 [0.39, 0.94]	0.91 [0.91, 0.92]

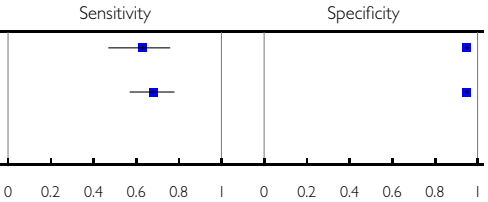


Test 58. Age, 1T total hCG and 1T PAPP-A, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 58 Age, 1T total hCG and 1T PAPP-A, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Haddow 1998	30	158	18	3011	0.63 [0.47, 0.76]	0.95 [0.94, 0.96]
Wald 2003a	58	51	27	974	0.68 [0.57, 0.78]	0.95 [0.94, 0.96]



Test 59. Age, 1T PAPP-A and 1T Inhibin, risk 1:100.

Review: First trimester serum tests for Down's syndrome screening

Test: 59 Age, 1T PAPP-A and 1T Inhibin, risk 1:100

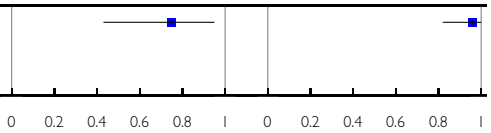
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	8	1	4	28	0.67 [0.35, 0.90]	0.97 [0.82, 1.00]		

Test 60. Age, 1T PAPP-A and 1T Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 60 Age, 1T PAPP-A and 1T Inhibin, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	9	1	3	27	0.75 [0.43, 0.95]	0.96 [0.82, 1.00]		

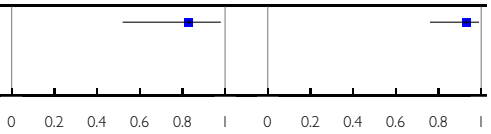


Test 61. Age, 1T PAPP-A and 1T Inhibin, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

Test: 61 Age, 1T PAPP-A and 1T Inhibin, risk 1:400

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	10	2	2	26	0.83 [0.52, 0.98]	0.93 [0.76, 0.99]		

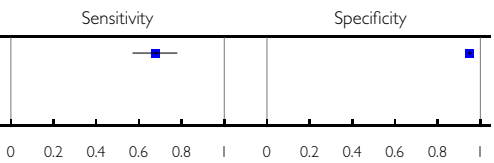


Test 62. Age, 1T PAPP-A and 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 62 Age, 1T PAPP-A and 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	58	51	27	974	0.68 [0.57, 0.78]	0.95 [0.94, 0.96]

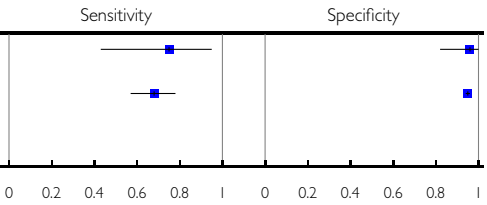


Test 63. Age, 1T PAPP-A and 1T Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 63 Age, 1T PAPP-A and 1T Inhibin, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	9	1	3	27	0.75 [0.43, 0.95]	0.96 [0.82, 1.00]
Wald 2003a	58	51	27	974	0.68 [0.57, 0.78]	0.95 [0.94, 0.96]

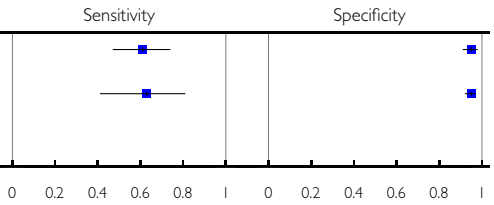


Test 64. Age, 1T PAPP-A and 1T ITA, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 64 Age, 1T PAPP-A and 1T ITA, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Spencer 2002a	33	11	21	213	0.61 [0.47, 0.74]	0.95 [0.91, 0.98]
Weinans 2005	15	16	9	304	0.63 [0.41, 0.81]	0.95 [0.92, 0.97]

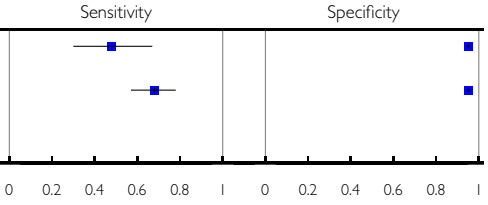


Test 65. Age, 1T PAPP-A and 1T AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 65 Age, 1T PAPP-A and 1T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Tsukerman 1999	15	78	16	1486	0.48 [0.30, 0.67]	0.95 [0.94, 0.96]
Wald 2003a	58	51	27	974	0.68 [0.57, 0.78]	0.95 [0.94, 0.96]

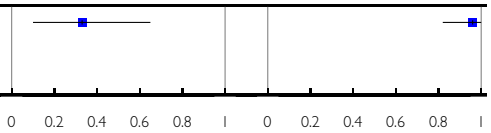


Test 66. Age, 1T free ßhCG and 1T Inhibin, risk 1:100.

Review: First trimester serum tests for Down's syndrome screening

Test: 66 Age, 1T free hCG and 1T Inhibin, risk 1:100

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	4	1	8	27	0.33 [0.10, 0.65]	0.96 [0.82, 1.00]		

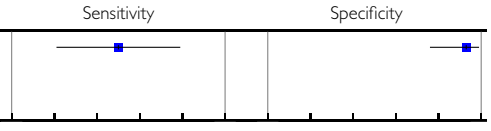


Test 67. Age, 1T free ßhCG and 1T Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 67 Age, 1T free hCG and 1T Inhibin, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	6	2	6	26	0.50 [0.21, 0.79]	0.93 [0.76, 0.99]		

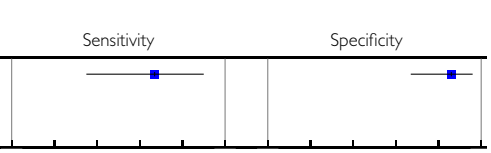


Test 68. Age, 1T free ßhCG and 1T Inhibin, risk 1:400.

Review: First trimester serum tests for Down's syndrome screening

Test: 68 Age, 1T free hCG and 1T Inhibin, risk 1:400

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	8	4	4	24	0.67 [0.35, 0.90]	0.86 [0.67, 0.96]		



Test 69. Age, 1T free ßhCG and 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 69 Age, 1T free hCG and 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003a	37	51	48	974	0.44 [0.33, 0.55]	0.95 [0.94, 0.96]		

Test 70. Age, 1T free ßhCG and 1T Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 70 Age, 1T free hCG and 1T Inhibin, mixed cut-points

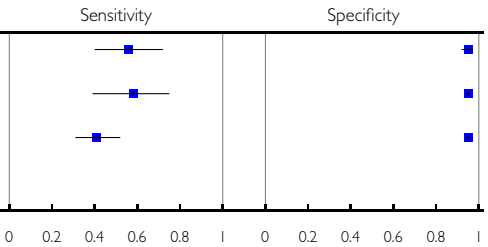
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2005	6	2	6	26	0.50 [0.21, 0.79]	0.93 [0.76, 0.99]		
Wald 2003a	37	51	48	974	0.44 [0.33, 0.55]	0.95 [0.94, 0.96]		

Test 71. Age, 1T free ßhCG and 1T AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 71 Age, 1T free hCG and 1T AFP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	23	12	18	234	0.56 [0.40, 0.72]	0.95 [0.92, 0.97]
Tsukerman 1999	18	78	13	1486	0.58 [0.39, 0.75]	0.95 [0.94, 0.96]
Wald 2003a	35	51	50	974	0.41 [0.31, 0.52]	0.95 [0.94, 0.96]

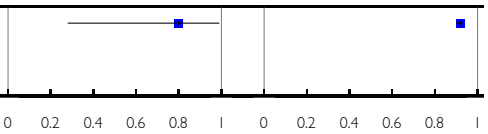


Test 72. Age, 1T free ßhCG and 1T AFP, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 72 Age, 1T free hCG and 1T AFP, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Benattar 1999	4	137	1	1514	0.80 [0.28, 0.99]	0.92 [0.90, 0.93]

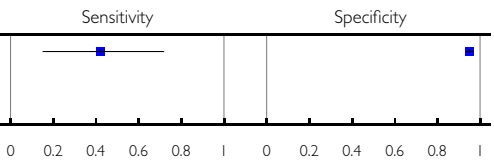


Test 73. Age, 1T free ßhCG and 1T AFP, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 73 Age, 1T free hCG and 1T AFP, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	5	25	7	475	0.42 [0.15, 0.72]	0.95 [0.93, 0.97]

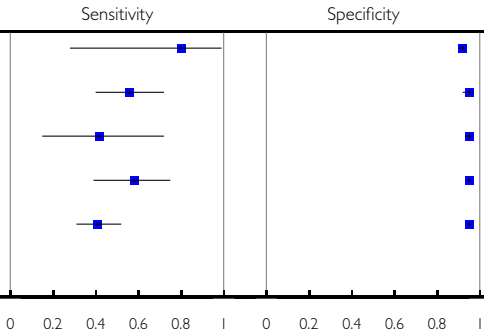


Test 74. Age, 1T free ßhCG and 1T AFP, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 74 Age, 1T free hCG and 1T AFP, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Benattar 1999	4	137	1	1514	0.80 [0.28, 0.99]	0.92 [0.90, 0.93]
Biagiotti 1995	23	12	18	234	0.56 [0.40, 0.72]	0.95 [0.92, 0.97]
Forest 1995	5	25	7	475	0.42 [0.15, 0.72]	0.95 [0.93, 0.97]
Tsukerman 1999	18	78	13	1486	0.58 [0.39, 0.75]	0.95 [0.94, 0.96]
Wald 2003a	35	51	50	974	0.41 [0.31, 0.52]	0.95 [0.94, 0.96]

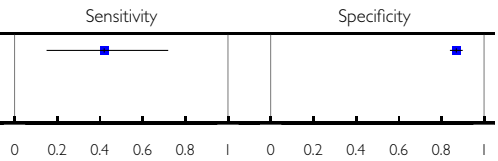


Test 75. Age, 1T AFP and 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 75 Age, 1T AFP and 1T uE3, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	5	66	7	434	0.42 [0.15, 0.72]	0.87 [0.84, 0.90]

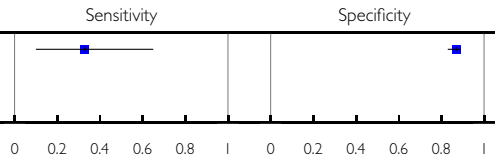


Test 76. Age, 1T AFP and 1T free αhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 76 Age, 1T AFP and 1T free α hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	4	67	8	433	0.33 [0.10, 0.65]	0.87 [0.83, 0.89]

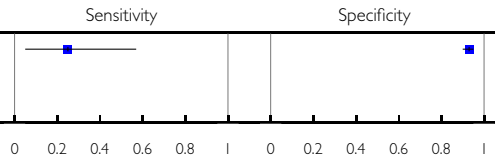


Test 77. Age, 1T free ßhCG and 1T total hCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 77 Age, 1T free hCG and 1T total hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	3	37	9	463	0.25 [0.05, 0.57]	0.93 [0.90, 0.95]

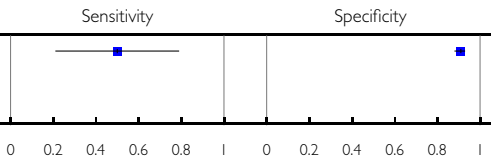


Test 78. Age, 1T free ßhCG and 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 78 Age, 1T free hCG and 1T uE3, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	6	45	6	455	0.50 [0.21, 0.79]	0.91 [0.88, 0.93]

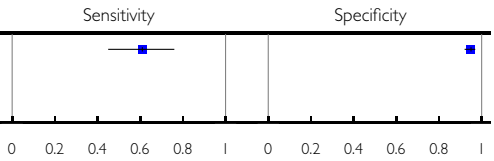


Test 79. Age, 1T free ßhCG and 1T uE3, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 79 Age, 1T free hCG and 1T uE3, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	25	12	16	234	0.61 [0.45, 0.76]	0.95 [0.92, 0.97]

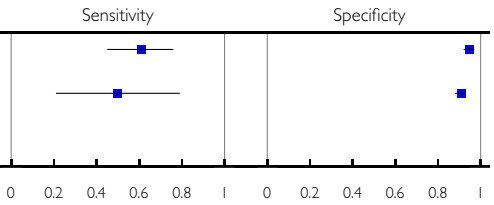


Test 80. Age, 1T free ßhCG and 1T uE3, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 80 Age, 1T free hCG and 1T uE3, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	25	12	16	234	0.61 [0.45, 0.76]	0.95 [0.92, 0.97]
Forest 1995	6	45	6	455	0.50 [0.21, 0.79]	0.91 [0.88, 0.93]

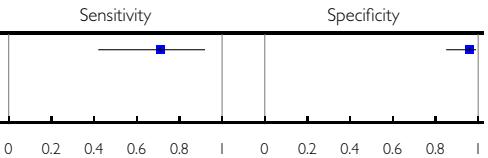


Test 81. Age, 1T free ßhCG and 1T SP1, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 81 Age, 1T free hCG and 1T SP1, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2004	10	2	4	44	0.71 [0.42, 0.92]	0.96 [0.85, 0.99]

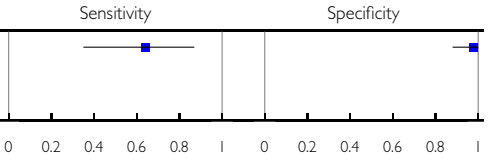


Test 82. Age, 1T free ßhCG and 1T SP1 risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 82 Age, 1T free hCG and 1T SP1 risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2004	9	1	5	45	0.64 [0.35, 0.87]	0.98 [0.88, 1.00]

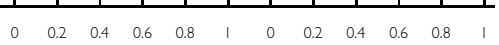


Test 83. Age, 1T AFP and 1T total hCG, 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 83 Age, 1T AFP and 1T total hCG, 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	4	36	8	464	0.33 [0.10, 0.65]	0.93 [0.90, 0.95]		

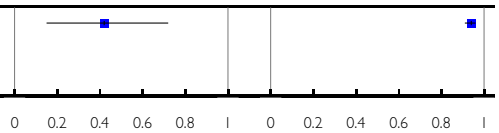


Test 84. Age, 1T free ßhCG and 1T free αhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 84 Age, 1T free hCG and 1T free α hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	5	31	7	469	0.42 [0.15, 0.72]	0.94 [0.91, 0.96]		



Test 85. Age, 1T total hCG and 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 85 Age, 1T total hCG and 1T uE3, risk 1:384

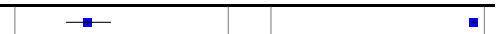
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	5	41	7	459	0.42 [0.15, 0.72]	0.92 [0.89, 0.94]		

Test 86. Age, 1T total hCG and 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 86 Age, 1T total hCG and 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003a	29	51	56	974	0.34 [0.24, 0.45]	0.95 [0.94, 0.96]		



Test 87. Age, 1T total hCG and 1T free αhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 87 Age, 1T total hCG and 1T free α hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	5	39	7	461	0.42 [0.15, 0.72]	0.92 [0.89, 0.94]		

Test 88. Age, 1T uE3 and 1T free αhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 88 Age, 1T uE3 and 1T free α hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	4	56	8	444	0.33 [0.10, 0.65]	0.89 [0.86, 0.91]		

Test 89. Age, 1T PAPP-A, 1T free ßhCG and 1T AFP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 89 Age, 1T PAPP-A, 1T free hCG and 1T AFP, 5FPR

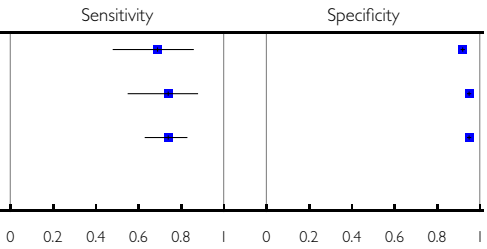
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Tsukerman 1999	23	78	8	1486	0.74 [0.55, 0.88]	0.95 [0.94, 0.96]		
Wald 2003a	63	51	22	974	0.74 [0.63, 0.83]	0.95 [0.94, 0.96]		

Test 90. Age, 1T PAPP-A, 1T free ßhCG and 1T AFP, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 90 Age, 1T PAPP-A, 1T free hCG and 1T AFP, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Muller 2003a	18	437	8	5020	0.69 [0.48, 0.86]	0.92 [0.91, 0.93]
Tsukerman 1999	23	78	8	1486	0.74 [0.55, 0.88]	0.95 [0.94, 0.96]
Wald 2003a	63	51	22	974	0.74 [0.63, 0.83]	0.95 [0.94, 0.96]

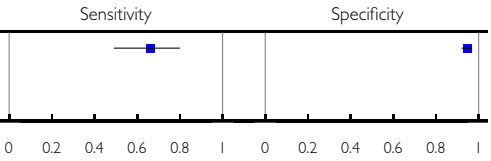


Test 91. Age, 1T free ßhCG, 1T AFP and 1T uE3, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 91 Age, 1T free hCG, 1T AFP and 1T uE3, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	27	12	14	234	0.66 [0.49, 0.80]	0.95 [0.92, 0.97]

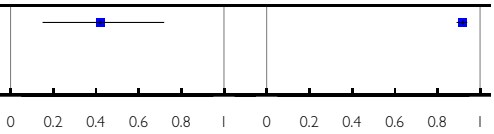


Test 92. Age, 1T free ßhCG, 1T AFP and 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 92 Age, 1T free hCG, 1T AFP and 1T uE3, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	5	40	7	460	0.42 [0.15, 0.72]	0.92 [0.89, 0.94]

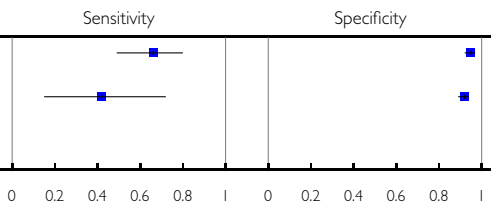


Test 93. Age, 1T free ßhCG, 1T AFP and 1T uE3, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 93 Age, 1T free hCG, 1T AFP and 1T uE3, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Biagiotti 1995	27	12	14	234	0.66 [0.49, 0.80]	0.95 [0.92, 0.97]
Forest 1995	5	40	7	460	0.42 [0.15, 0.72]	0.92 [0.89, 0.94]

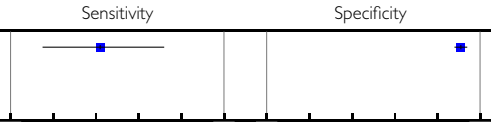


Test 94. Age, 1T total hCG, 1T AFP and 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 94 Age, 1T total hCG, 1T AFP and 1T uE3, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	5	44	7	456	0.42 [0.15, 0.72]	0.91 [0.88, 0.94]

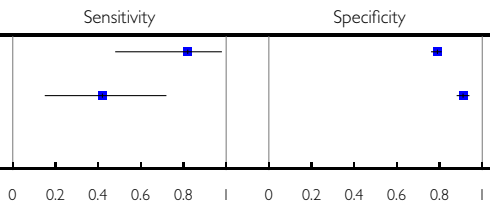


Test 95. Age, 1T total hCG, 1T AFP and 1T uE3, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 95 Age, 1T total hCG, 1T AFP and 1T uE3, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Crandall 1993	9	208	2	774	0.82 [0.48, 0.98]	0.79 [0.76, 0.81]
Forest 1995	5	44	7	456	0.42 [0.15, 0.72]	0.91 [0.88, 0.94]

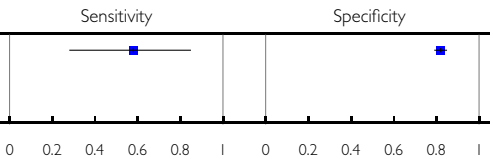


Test 96. Age, 1T AFP, free αhCG and 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 96 Age, 1T AFP, free α hCG and 1T uE3, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	7	89	5	411	0.58 [0.28, 0.85]	0.82 [0.79, 0.85]

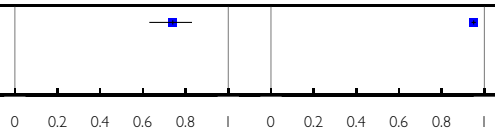


Test 97. Age, 1T PAPP-A, 1T free ßhCG and 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 97 Age, 1T PAPP-A, 1T free hCG and 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003a	63	51	22	974	0.74 [0.63, 0.83]	0.95 [0.94, 0.96]		

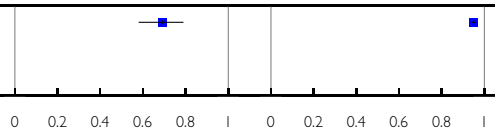


Test 98. Age, 1T PAPP-A, 1T total hCG and 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 98 Age, 1T PAPP-A, 1T total hCG and 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003a	59	51	26	974	0.69 [0.58, 0.79]	0.95 [0.94, 0.96]		

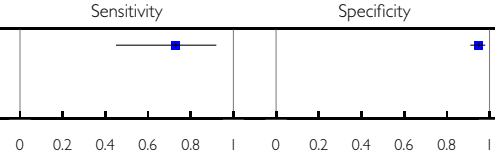


Test 99. Age, 1T PAPP-A, sp1 and 1T ProMBP, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 99 Age, 1T PAPP-A, sp1 and 1T ProMBP, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2004	11	9	4	168	0.73 [0.45, 0.92]	0.95 [0.91, 0.98]		

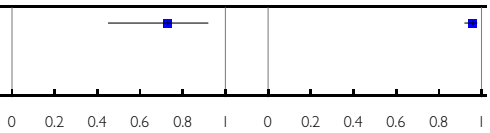


Test 100. Age, 1T PAPP-A, sp1 and 1T ProMBP, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 100 Age, 1T PAPP-A, sp1 and 1T ProMBP, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2004	11	7	4	170	0.73 [0.45, 0.92]	0.96 [0.92, 0.98]		

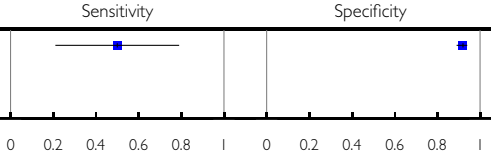


Test 101. Age, 1T free ßhCG, 1T total hCG, 1T AFP and 1T uE3, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 101 Age, 1T free hCG, 1T total hCG, 1T AFP and 1T uE3, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	6	41	6	459	0.50 [0.21, 0.79]	0.92 [0.89, 0.94]		

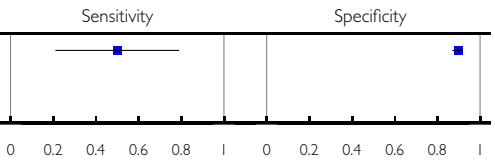


Test 102. Age, 1T total hCG, 1T AFP, 1T uE3 and 1T free αhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 102 Age, 1T total hCG, 1T AFP, 1T uE3 and 1T free α hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	6	51	6	449	0.50 [0.21, 0.79]	0.90 [0.87, 0.92]

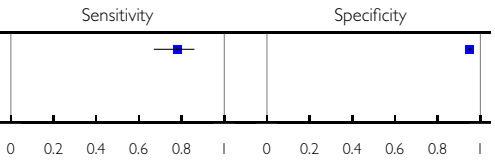


Test 103. Age, 1T PAPP-A, 1T free ßhCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 103 Age, 1T PAPP-A, 1T free hCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	66	51	19	974	0.78 [0.67, 0.86]	0.95 [0.94, 0.96]

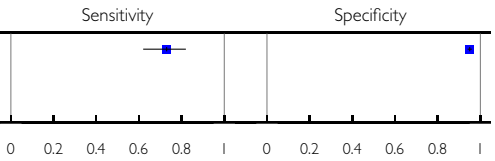


Test 104. Age, 1T PAPP-A, 1T total hCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 104 Age, 1T PAPP-A, 1T total hCG, 1T AFP, 1T uE3 and 1T Inhibin, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	62	51	23	974	0.73 [0.62, 0.82]	0.95 [0.94, 0.96]



Test 105. Age, 1T free ßhCG, 1T total hCG, 1T AFP, 1T uE3 and 1T free αhCG, risk 1:384.

Review: First trimester serum tests for Down's syndrome screening

Test: 105 Age, 1T free hCG, 1T total hCG, 1T AFP, 1T uE3 and 1T free α hCG, risk 1:384

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	4	51	8	449	0.33 [0.10, 0.65]	0.90 [0.87, 0.92]		

Test 106. Age, 1T hPL, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 106 Age, 1T hPL, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2007a	21	9	26	127	0.45 [0.30, 0.60]	0.93 [0.88, 0.97]		

Test 107. Age, 1T hPL, 1T PAPP-A, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 107 Age, 1T hPL, 1T PAPP-A, risk 1:250

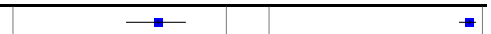
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2007a	26	8	21	128	0.55 [0.40, 0.70]	0.94 [0.89, 0.97]		

Test 108. Age, 1T hPL, 1T free ßhCG, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 108 Age, 1T hPL, 1T free hCG, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2007a	32	8	15	128	0.68 [0.53, 0.81]	0.94 [0.89, 0.97]		



Test 109. Age, 1T hPL, 1T PAPP-A, 1T free ßhCG, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 109 Age, 1T hPL, 1T PAPP-A, 1T free hCG, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2007a	36	7	11	129	0.77 [0.62, 0.88]	0.95 [0.90, 0.98]		

Test 110. Age, 1T PGH, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 110 Age, 1T PGH, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2009	30	15	44	246	0.41 [0.29, 0.53]	0.94 [0.91, 0.97]		

Test 111. Age, 1T PGH, 1T PAPP-A , risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 111 Age, 1T PGH, 1T PAPP-A , risk 1:250

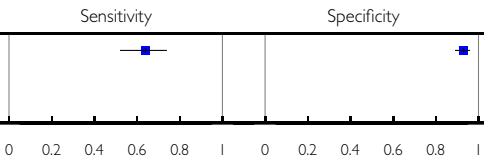
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2009	48	18	26	243	0.65 [0.53, 0.76]	0.93 [0.89, 0.96]		

Test 112. Age, 1T PGH, 1T free ßhCG , risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 112 Age, 1T PGH, 1T free hCG , risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2009	47	19	27	242	0.64 [0.52, 0.74]	0.93 [0.89, 0.96]

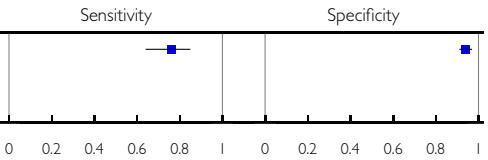


Test 113. Age, 1T PGH, 1T PAPP-A, 1T free ßhCG , risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 113 Age, 1T PGH, 1T PAPP-A, 1T free hCG , risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2009	56	15	18	246	0.76 [0.64, 0.85]	0.94 [0.91, 0.97]

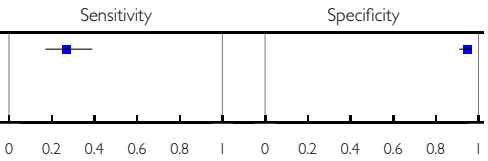


Test 114. Age, 1T GHBP, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 114 Age, 1T GHBP, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2009	20	14	54	247	0.27 [0.17, 0.39]	0.95 [0.91, 0.97]

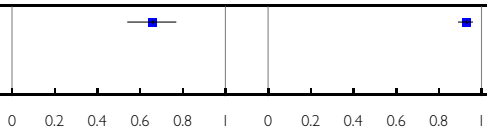


Test 115. Age, 1T GHBP, 1T PAPP-A, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 115 Age, 1T GHBP, 1T PAPP-A, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2009	49	18	25	243	0.66 [0.54, 0.77]	0.93 [0.89, 0.96]		

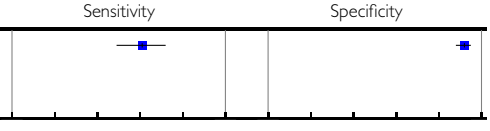


Test 116. Age, 1T GHBP, 1T free ßhCG, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 116 Age, 1T GHBP, 1T free hCG, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2009	45	22	29	239	0.61 [0.49, 0.72]	0.92 [0.88, 0.95]		



Test 117. Age, 1T GHBP, 1T PGH, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 117 Age, 1T GHBP, 1T PGH, risk 1:250

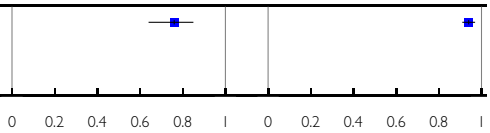
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2009	35	17	39	244	0.47 [0.36, 0.59]	0.93 [0.90, 0.96]		

Test 118. Age, 1T GHBP, 1T PAPP-A, 1T free ßhCG , risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 118 Age, 1T GHBP, 1T PAPP-A, 1T free hCG , risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2009	56	15	18	246	0.76 [0.64, 0.85]	0.94 [0.91, 0.97]		

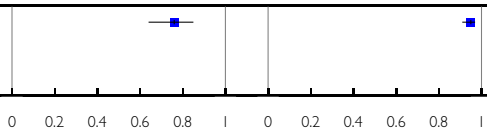


Test 119. Age, 1T GHBP, 1T PGH, 1T PAPP-A, 1T free ßhCG , risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 119 Age, 1T GHBP, 1T PGH, 1T PAPP-A, 1T free hCG , risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Christiansen 2009	56	14	18	247	0.76 [0.64, 0.85]	0.95 [0.91, 0.97]		

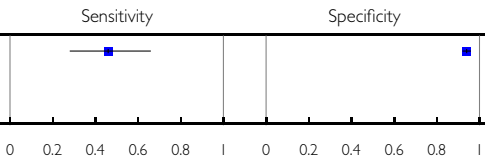


Test 120. Age, 1T ADAM 12, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 120 Age, 1T ADAM 12, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2010	13	30	15	473	0.46 [0.28, 0.66]	0.94 [0.92, 0.96]

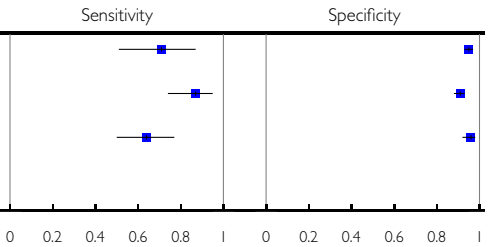


Test 121. Age, 1T ADAM 12, 1T PAPP-A, 1T free ßhCG, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 121 Age, 1T ADAM 12, 1T PAPP-A, 1T free hCG, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2010	20	24	8	479	0.71 [0.51, 0.87]	0.95 [0.93, 0.97]
Torring 2010	40	60	6	585	0.87 [0.74, 0.95]	0.91 [0.88, 0.93]
Valinen 2009	34	10	19	216	0.64 [0.50, 0.77]	0.96 [0.92, 0.98]

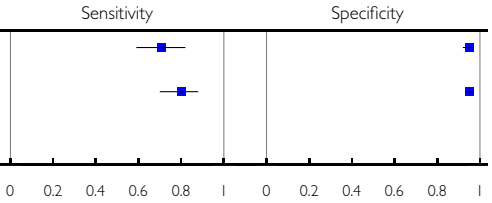


Test 122. Age, PlGF, 1T PAPP-A, 1T free ßhCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 122 Age, PlGF, 1T PAPP-A, 1T free hCG, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Cowans 2010	50	19	20	356	0.71 [0.59, 0.82]	0.95 [0.92, 0.97]
Zaragoza 2009	72	30	18	579	0.80 [0.70, 0.88]	0.95 [0.93, 0.97]

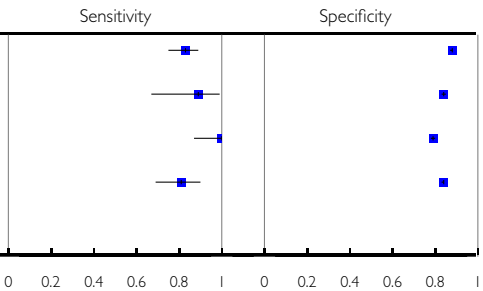


Test 123. Age, 1T PAPP-A and 1T free ßhCG, risk 1:300.

Review: First trimester serum tests for Down's syndrome screening

Test: 123 Age, 1T PAPP-A and 1T free hCG, risk 1:300

Study	TP	FP	FN	TN	Sensitivity	Specificity
Kagan 2009	101	2373	21	17241	0.83 [0.75, 0.89]	0.88 [0.87, 0.88]
Kozlowski 2007 GC	17	1081	2	5806	0.89 [0.67, 0.99]	0.84 [0.83, 0.85]
Kozlowski 2007 PC	26	802	0	3034	1.00 [0.87, 1.00]	0.79 [0.78, 0.80]
Schaelike 2009	48	1730	11	8879	0.81 [0.69, 0.90]	0.84 [0.83, 0.84]

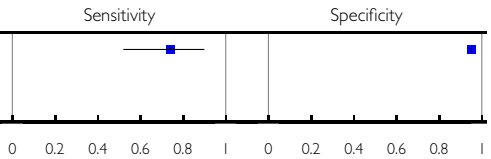


Test 124. Age, 1T PAPP-A, 1T Hyperglycosylated hCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 124 Age, 1T PAPP-A, 1T Hyperglycosylated hCG, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Palomaki 2007	17	538	6	10214	0.74 [0.52, 0.90]	0.95 [0.95, 0.95]

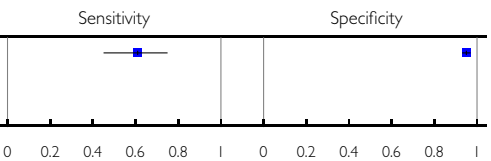


Test 128. Age, ADAM 12, 1T PAPP-A, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 128 Age, ADAM 12, 1T PAPP-A, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Torring 2010	28	32	18	613	0.61 [0.45, 0.75]	0.95 [0.93, 0.97]

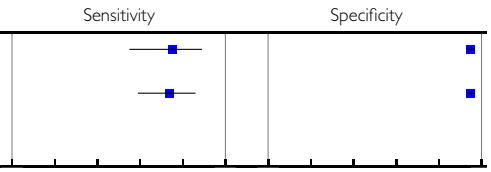


Test 129. Age, ADAM 12, 1T PAPP-A, 1T free ßhCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 129 Age, ADAM 12, 1T PAPP-A, 1T free hCG, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2010	21	25	7	478	0.75 [0.55, 0.89]	0.95 [0.93, 0.97]
Torring 2010	34	32	12	613	0.74 [0.59, 0.86]	0.95 [0.93, 0.97]



Test 130. Age, 1T PlGF, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 130 Age, 1T PlGF, 5FPR

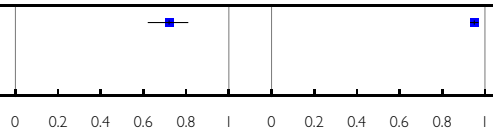
Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Zaragoza 2009	39	30	51	579	0.43 [0.33, 0.54]	0.95 [0.93, 0.97]		

Test 131. 1T PlGF, 1T PAPP-A, 1T free ßhCG, 5FPR.

Review: First trimester serum tests for Down's syndrome screening

Test: 131 1T PlGF, 1T PAPP-A, 1T free hCG, 5FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Zaragoza 2009	65	30	25	579	0.72 [0.62, 0.81]	0.95 [0.93, 0.97]		

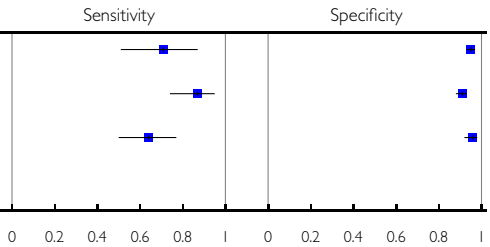


Test 132. Age, 1T ADAM 12, 1T PAPP-A, 1T free ßhCG, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 132 Age, 1T ADAM 12, 1T PAPP-A, 1T free hCG, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2010	20	24	8	479	0.71 [0.51, 0.87]	0.95 [0.93, 0.97]
Torring 2010	40	60	6	585	0.87 [0.74, 0.95]	0.91 [0.88, 0.93]
Valinen 2009	34	10	19	216	0.64 [0.50, 0.77]	0.96 [0.92, 0.98]

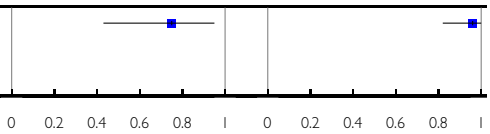


Test 133. Age, 1T PAPP-A, 1T free ßhCG and 1T Inhibin, risk 1:250.

Review: First trimester serum tests for Down's syndrome screening

Test: 133 Age, 1T PAPP-A, 1T free hCG and 1T Inhibin, risk 1:250

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	9	1	3	27	0.75 [0.43, 0.95]	0.96 [0.82, 1.00]

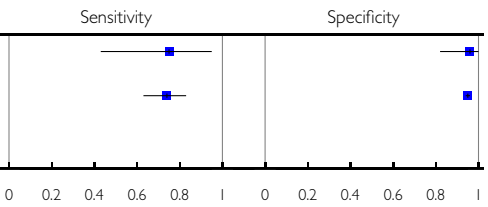


Test 134. Age, 1T PAPP-A, 1T free ßhCG, and 1T Inhibin, mixed cut-points.

Review: First trimester serum tests for Down's syndrome screening

Test: 134 Age, 1T PAPP-A, 1T free hCG, and 1T Inhibin, mixed cut-points

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2005	9	1	3	27	0.75 [0.43, 0.95]	0.96 [0.82, 1.00]
Wald 2003a	63	51	22	974	0.74 [0.63, 0.83]	0.95 [0.94, 0.96]



ADDITIONAL TABLES

Table 1. Direct comparisons of the sensitivity of nine test strategies at the 5% false positive rate

Ratio of sensitivity (95% CI), P value for comparison (studies)	Free ßhCG	PAPP-A	Age, free ßhCG	Age, PAPP-A	Age, PAPP-A , free ßhCG	Age, free ßhCG, AFP	Age, ADAM 12, PAPP-A, free ßhCG	Age, PAPP-A, free ßhCG, AFP
PAPP-A	1.78 (1.10 to 2.88), P = 0.02 (2)							
Age, free ßhCG	1.67 (1.11 to 2.50). P = 0.013 (2)	0.94 (0.68 to 1.29), P = 0.70 (2)						
Age, PAPP-A	2.15 (1.37 to 3.38), P = 0.001 (2)	1.20 (0.86 to 1.67), P = 0.29 (3)	1.26 (1.02 to 1.57), P = 0.034 (4)					
Age, PAPP-A , free ßhCG	2.62 (1.77 to 3.87), P < 0.001 (2)	1.47 (1.09 to 2.00), P = 0.012 (2)	1.61 (1.31 tp 1.98), P < 0.001 (5)	1.26 (1.04 to 1.52), P = 0.02 (4)				

Table 1. Direct comparisons of the sensitivity of nine test strategies at the 5% false positive rate (*Continued*)

Age, free ßhCG, AFP	2.19 (1.31 to 3.64), P = 0.002 (1)	0.71 (0.52 to 0.98), P = 0.03 (1)	1.08 (0.80 to 1.46), P = 0.62 (2)	0.61 (0.46 to 0.82), P < 0.001 (1)	0.63 (0.47 to 0.86), P = 0.004 (2)			
Age, ADAM 12, PAPP-A, free ßhCG	-	-	-	-	1.04 (0.85 to 1.26), P = 0.71 (2)	-		
Age, PAPP-A, free ßhCG, AFP	3.94 (2.49 to 6.23), P < 0.001 (1)	1.29 (1.03 to 1.60), P = 0.024 (1)	1.91 (1.42 to 2.56), P < 0.001 (1)	1.11 (0.91 to 1.34), P = 0.31 (1)	1.02 (0.88 to 1.20), P = 0.77 (2)	1.62 (1.19 to 2.19), P = 0.002 (2)	-	
Age, PlGF, PAPP-A, free ßhCG	-	-	-	-	1.03 (0.91 to 1.17), P = 0.61 (2)	-	-	-

- indicates that no comparative study was available for the pair of tests.

Direct comparisons were made only using data from studies which compared each pair of tests on the same women. Where there were at least two studies, meta-analysis was performed to summarise and compare the sensitivities. The ratio of sensitivities was computed by division of the sensitivity for the column by the sensitivity for the row. If the ratio of sensitivity is greater than one then the sensitivity of the test for the column is higher than that for the row, if less than one the sensitivity of the test in the row is higher than in the column. All test comparisons that were evaluated by only one study were from Wald 2003. The ratio of the sensitivities for test comparisons from a single study were calculated as a ratio of two proportions.

ADAM12: a disintegrin and metalloprotease; **AFP:** alpha-fetoprotein; **ßhCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **PAPP-A:** pregnancy-associated plasma protein A; **PIGF:** placental growth factor; **PROMBP:** proform of eosinophil major basic protein

Table 2. Indirect comparisons of the sensitivity of nine test strategies at the 5% false positive rate

Ratio of sensitivity (95% CI), P value for comparison			**Free ßhCG**	**PAPP-A**	**Age, free ßhCG**	**Age, PAPP-A**	**Age, PAPP-A, free ßhCG**	**Age, free ßhCG, AFP**	**Age, ADAM 12, PAPP-A, free ßhCG**	**Age, PAPP-A, free ßhCG, AFP**
		Studies (cases/ women)	4 (390/ 4280)	4 (325/ 2837)	7 (460/ 5893)	5 (359/ 3491)	17 (1037/ 49827)	3 (157/ 2992)	2 (74/ 1222)	2 (116/ 2705)
	Studies (cases/	**Sensitivity %**	25 (18 to 34)	52 (39 to 65)	42 (36 to 48)	55 (46 to 63)	68 (65 to 71)	49 (39 to 60)	74 (63 to 83)	74 (65 to

Table 2. Indirect comparisons of the sensitivity of nine test strategies at the 5% false positive rate (*Continued*)

	women)	**(95% CI)**								81)
PAPP-A	4 (325/ 2837)	52 (39 to 65)	2.05 (1.37 to 3.09), P = 0.001							
Age, free ßhCG	7 (460/ 5893)	42 (36 to 48)	1.66 (1.17 to 2.36), P = 0.004	0.81 (0.61 to 1.08), P = 0.15						
Age, PAPP-A	5 (359/ 3491)	55 (46 to 63)	2.16 (1.51 to 3.10), P < 0.001	1.05 (0.78 to 1.42), P = 0.73	1.30 (1.05 to 1.61), P = 0.015					
Age, PAPP-A, free ßhCG	17 (1037/ 49827)	68 (65 to 71)	2.70 (1.95 to 3.73), P < 0.001	1.31 (1.02 to 1.70), P = 0.037	1.62 (1.40 to 1.88), P < 0.001	1.25 (1.05 to 1.47), P = 0.01				
Age, free ßhCG, AFP	3 (157/ 2992)	49 (39 to 60)	1.95 (1.33 to 2.86), P = 0.001	0.95 (0.69 to 1.32), P = 0.76	1.18 (0.92 to 1.51), P = 0.20	0.90 (0.69 to 1.17), P = 0.45	0.72 (0.59 to 0.89), P = 0.003			
Age, ADAM 12, PAPP-A, free ßhCG	2 (74/ 1222)	74 (63 to 83)	2.94 (2.07 to 4.16), P < 0.001	1.43 (1.07 to 1.90), P = 0.014	1.77 (1.46 to 2.14), P < 0.001	1.36 (1.10 to 1.67), P = 0.004	1.09 (0.95 to 1.25), P = 0.24	1.50 (1.17 to 1.92), P = 0.001		
Age, PAPP-A, free ßhCG, AFP	2 (116/ 2705)	74 (65 to 81)	2.93 (2.09 to 4.11), P < 0.001	1.43 (1.08 to 1.88), P = 0.011	1.76 (1.48 to 2.10), P < 0.001	1.35 (1.11 to 1.64), P = 0.002	1.09 (0.97 to 1.22), P = 0.16	1.50 (1.19, to 1.89). P = 0.001	1.00 (0.84 to 1.18), P = 0.98	
Age, PlGF, PAPP-A, free ßhCG	2 (160/ 1144)	76 (69 to 82)	3.01 (2.16 to 4.20), P < 0.001	1.47 (1.12 to 1.91), P = 0.005	1.81 (1.54 to 2.14), P < 0.001	1.39 (1.16 to 1.67), P < 0.001	1.12 (1.01 to 1.23), P = 0.024	1.54 (1.23 to 1.93), P < 0.001	1.03 (0.87 to 1.20), P = 0.75	1.03 (0.90 to 1.18), P = 0.7

Ratio of sensitivities were computed by division of the sensitivity for the column by the sensitivity for the row. If the ratio of sensitivity is greater than one then the sensitivity of the test for the column is higher than that for the row, if less than one the sensitivity of the test in the row is higher than in the column.

AFP: alpha-fetoprotein; αhCG: alpha human chorionic gonadotrophin; **ßhCG:** beta human chorionic gonadotrophin; **CI:** confidence interval; **PAPP-A:** Pregnancy-associated plasma protein A

Table 3. Summary of study characteristics

Study	PAPP-A, free ßhCG and age*	Maternal age (years)	Reference standard	Population	Study design	Study location
Baviera 2010		Mean 35.3 for Down's cases, 30.4 for control	Amniocentesis or follow-up to birth	Routine screening	Case-control	Italy
Benattar 1999		Mean 32 (16-46), 8.3% > 35	Amniocentesis due to maternal age > 38 years (6.1% or women). Karyotyping encouraged for women with positive result on one or more index test. No details of reference standard for index test negative women	Routine screening	Prospective cohort	France
Biagiotti 1995		Not reported	Amniocentesis or CVS	High-risk referral for invasive testing	Case-control	Italy
Biagiotti 1998	X	Unclear (maybe all ≥ 38)	Amniocentesis or CVS	High-risk referral for invasive testing	Retrospective case-control	Italy
Brambati 1993		Median 38 (20-47)	CVS	High-risk referral for invasive testing	Retrospective cohort	Italy
Brambati 1994	X	Not reported	CVS	High-risk referral for invasive testing	Case-control	Italy
Brameld 2008		Median 31 (14-47), 20% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Australia
Brizot 1994		Median 38 (22-45)	Fetal karyotyping	High-risk referral for invasive testing	Retrospective case-control	UK

Table 3. Summary of study characteristics (*Continued*)

Casals 1996		94.4% > 35	CVS	High-risk referral for invasive testing	Retrospective case-control	Spain
Christiansen 1999		Not reported	Karyotyping	High-risk referral for invasive testing	Case-control	Denmark
Christiansen 2004		Not reported	CVS (for 120 of cases of Down's) or follow-up to birth (for 36 of cases of Down's)	Routine screening	Case-control	Denmark
Christiansen 2005		Not reported	Karyotyping	Screening programmes for syphilis and Down's syndrome	Case-control	Denmark
Christiansen 2007a	X	Median 37.7 (24-48) for Down's cases, 36.4 (22-44) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Christiansen 2009	X	Median 37.5 for Down's cases, 36.4 for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Christiansen 2010	X	Median 36 (25-44) for Down's cases, 29 (17-45) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Cowans 2010	X	Mean 37.0 (IQR 32.9-40.5) for Down's cases, 32.4 (IQR 29.0-35.9) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	UK
Crandall 1993		90% > 35	Amniocentesis	High-risk referral for invasive testing	Retrospective cohort	USA
Crossley 2002a		Median 29.9, 15.4% ≥ 35	CVS offered where women had high NT measurements.	Routine screening	Prospective cohort	UK

Table 3. Summary of study characteristics (*Continued*)

			Also amniocentesis or follow-up to birth			
De Graaf 1999a	X	Not reported	Amniocentesis or CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Forest 1995		Mean 29.1 (SD 4.7), 10.7% ≥ 35	Follow-up to birth	Routine screening	Case-control	Canada
Forest 1997	X	Mean 27.9, 10.7% ≥ 35	Follow-up to birth	Routine screening	Case-control	Canada
Gyselaers 2005		Not reported	Amniocentesis, CVS and postnatal karyotyping	Routine screening	Prospective cohort	Belgium
Haddow 1998	X	Median 37 (15-51)	Amniocentesis or CVS	High-risk referral for invasive testing	Prospective cohort	USA
Kagan 2009	X	Mean 35.4 (14.1-52.2)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	UK
Kornman 1998		Not reported	CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Kozlowski 2007 GC		Median 32 (15-48), 26.4% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Cohort	Germany
Kozlowski 2007 PC		Median 34 (14-46), 43.2% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Cohort	Germany
Krantz 2000		34.7% ≥ 35	Not reported	Routine screening	Prospective cohort	USA
Kratzer 1991		Missing	CVS	High-risk referral for invasive testing	Case-control	USA
Laigaard 2003		Not reported	Karyotyping, unclear reference standard for con-	Routine screening	Case-control	Denmark

Table 3. Summary of study characteristics (*Continued*)

			trols			
Macintosh 1993		Median 38 (27-40)	CVS	High-risk referral for invasive testing	Retrospective cohort	UK and Italy
Muller 2003a		Not reported	Invasive testing (offered to women with high NT measurement) or follow-up to birth	Routine screening	Retrospective cohort	France
Nebiolo 1990		Approximately 75% ≥ 35	CVS	High-risk referral for invasive testing	Retrospective cohort	Italy
Niemimaa 2001a		17.5% ≥35	Invasive testing (patients considered high-risk based on NT screening) or follow-up to birth	Routine screening	Prospective cohort	Finland
Noble 1995		Median 34 (15-47), 47% ≥ 35	Karyotyping performed (27%), ultrasound examination at 20 weeks (65%), or follow-up to birth (9%)	Routine screening in a high-risk population	Prospective cohort	UK
Noble 1997		Median 34 (15-47)	CVS, follow-up to birth not reported	Routine screening	Case-control	UK
O'Leary 2006		Median 31 (14-47), 20% ≥ 35 years	CVS or amniocentesis (women assessed to be high risk on screening) or follow-up to birth	Routine screening	Prospective cohort	Australia
Orlandi 1997		Range 15-46, 35% ≥ 35	Not reported	Routine screening	Prospective cohort	Italy

Table 3. Summary of study characteristics (*Continued*)

Palomaki 2007		Mean maternal age 32.3 years (SD 4.6 years)	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Canada
Qin 1997		Not reported	CVS, amniocentesis, karyotyping at birth, unclear reference standard for control	Routine screening	Case-control	Denmark
Sahota 2010	X	Median 33.1, 30.1% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	China
Schaelike 2009		31.0% ≥35	Karyotyping or follow-up to birth	Routine screening	Prospective cohort	Germany
Scott 2004		Median 32 (15-44), 29% ≥ 35	Invasive testing or follow-up to birth	Routine screening	Prospective cohort	Australia
Spencer 1999a	X	Median cases 38 (19-46), controls 36 (15-47)	Invasive testing (high-risk women) or follow-up to birth	Referred for invasive testing or self-referred for screening	Case-control	UK
Spencer 2002a		Median cases 36 (20-44), controls 30 (16-41)	Not reported	Routine screening	Case-control	UK
Torring 2010	X	Mean 35 for Down's, 31 for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	Denmark
Tsukerman 1999	X	Not reported	Karyotyping, karyotyping at birth, follow-up to birth not reported	Routine screening	Case-control	Belarus
Valinen 2007		Mean 29.6, 18.6% ≥ 35	Karyotyping or follow-up to birth	Routine screening	Retrospective cohort	Finland
Valinen 2009		Not reported	Karyotyping or follow-up to birth	Routine screening	Case-control	Finland

Table 3. Summary of study characteristics (*Continued*)

Van Lith 1992		Not reported	CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Wald 2003a	X	Missing	Invasive testing (following second trimester screening) or follow-up to birth	Routine screening	Case-control	UK and Austria
Wallace 1995		Mean 32 (22-44) for Down's cases, 28 (19-38) for controls	Not reported	Routine screening	Case-control	UK
Wapner 2003	X	Mean 35 (SD 4.6), 50% ≥ 35	Invasive testing, miscarriage with cytogenetic testing, follow-up to birth	Routine screening	Prospective cohort	USA
Weinans 2005		Mean 38 (SD 2.7) for Down's cases, 37 (SD 3.0) for controls	CVS	High-risk referral for invasive testing	Case-control	The Netherlands
Wojdemann 2005		Mean 29, 10.8% ≥ 35	Invasive testing (in cases of increased risk) or follow-up to birth	Routine screening	Prospective cohort	Denmark
Zaragoza 2009	X	Median 37.9 (19.1-46.5) for Down's cases, 32.7 (16.1-45.2) for controls	Karyotyping or follow-up to birth	Routine screening	Case-control	UK

*The PAPP-A, free ßhCG and age test combination was the only test evaluated by at least 10 studies. X indicates that the test was evaluated in the study.

CVS: chorionic villus sampling; **IQR:** interquartile range; **SD:** standard deviation.

APPENDICES

Appendix 1. Search Strategy

Database: Ovid MEDLINE

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1 exp Prenatal Diagnosis/
2 nuchal translucency.mp.
3 exp Pregnancy-Associated Plasma Protein-A/
4 pregnancy associated plasma protein a.mp.
5 papp-a.mp.
6 exp Chorionic Gonadotropin, beta Subunit, Human/
7 (b-hcg or bhcg).mp.
8 human chorionic gonadotropin.mp.
9 exp alpha-Fetoproteins/
10 alphafetoprotein$.mp.
11 alpha-fetoprotein$.mp.
12 afp.mp.
13 (unconjugated estriol or unconjugated oestriol).mp.
14 ue3.mp.
15 exp INHIBINS/
16 inhibin a.mp.
17 ultrasound.mp.
18 amniocentesis/
19 chorion$ vill$ sampling.mp.
20 Chorionic Villi-Sampling/
21 nasal bone.mp.
22 tricuspid regurgitation.mp.
23 ductus venosus.mp
24 marker$.mp.
25 screen$.mp.
26 detect$.mp.
27 accura$.mp.
28 predict$.mp.
29 ROC.mp.
30 ROC curve/
31 AUC.mp.
32 Area under curve/
33 exp false negative reactions/ or exp false positive reactions/
34 (false positive$ or false negative$).mp.
35 likelihood ratio$.mp.
36 sensitiv$.mp.
37 specific$.mp.
38 diagnos$.ti,ab.
39 "reproducibility of results".mp.
40 reference value$.mp.
41 reference standard$.mp.
42 exp Down Syndrome/
43 downs syndrome.mp.
44 down syndrome.mp.
45 trisomy 21.mp.
46 Aneuploidy/
47 aneuploidy.mp.

48 Mosaicism/
49 mosaicism.mp.
50 or/1-41
51 or/42-49
52 50 and 51
53 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).mp.
54 52 and 53
55 animal/ not (humans/ and animal/)
56 54 not 55
****

Embase via Dialog Datastar

1. PRENATAL-DIAGNOSIS#.DE.
2. FETUS-ECHOGRAPHY#.DE.
3. PREGNANCY-ASSOCIATED-PLASMA-PROTEIN-A#.DE.
4. CHORIONIC-GONADOTROPIN-BETA-SUBUNIT#.DE.
5. HCG.AB.
6. PAPP.AB.
7. ALPHA-FETOPROTEIN#.DE.
8. AFP.AB.
9. ALPHA ADJ FETOPROTEIN$
10. ALPHAFETOPROTEIN$
11. BETA ADJ HUMAN ADJ CHORIONIC ADJ GONADOTROPIN
12. PREGNANCY ADJ ASSOCIATED ADJ PLASMA ADJ PROTEIN
13. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).TI.
14. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).AB.
15. UE3
16. INHIBIN-A#.DE.
17. INHIBIN ADJ A
18. ULTRASOUND
19. AMNIOCENTESIS
20. CHORION-VILLUS-SAMPLING.DE.
21. NASAL ADJ BONE
22. TRICUSPID ADJ REGURGITATION
23. DUCTUS ADJ VENOSUS
24. MARKER OR MARKERS
25. SCREEN OR SCREENING
26. DETECT OR DETECTING OR DETECTION
27. FALSE ADJ POSITIVE$
28. FALSE ADJ NEGATIVE$
29. SENSITIVITY OR SENSITIVE OR SENSITIVITIES
30. SPECIFICITY OR SPECIFICITIES
31. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).TI.
32. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).AB.
33. ROC.AB.
34. AUC.AB.
35. AREA-UNDER-THE-CURVE.DE.
36. ROC-CURVE.DE.
37. ACCURA$
38. PREDICT$
39. REPRODUCIBILITY.DE.

40. REFERENCE ADJ VALUE$
41. REFERENCE-VALUE.DE.
42. REFERENCE ADJ STANDARD$
43. DOWN-SYNDROME#.DE.
44. DOWN ADJ SYNDROME OR DOWNS ADJ SYNDROME
45. TRISOMY ADJ '21'
46. MOSAICISM
47. ANEUPLOIDY
48. ANTENATAL$ OR PRENATAL$ OR PREGNANCY OR PREGNANT OR TRIMESTER$ OR MATERNAL OR FETUS OR FOETUS OR FOETAL OR FETAL
49. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39 OR 40 OR 41 OR 42
50. 43 OR 44 OR 45 OR 46 OR 47
51. 48 AND 49 AND 50
52. HUMAN=YES
53. 51 AND 52

ADJ = adjacent AB = abstract
TI = title $ = truncation symbol DE = descriptor (similar to MeSH)
****

CINAHL via OVID

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1 exp Prenatal Diagnosis/
2 nuchal translucency.mp.
3 pregnancy associated plasma protein.mp.
4 papp$.ti,ab.
5 exp Gonadotropins, chorionic/
6 (b-hcg or bhcg).mp.
7 human chorionic gonadotropin.mp.
8 exp alpha-Fetoproteins/
9 alphafetoprotein$.mp.
10 alpha-fetoprotein$.mp.
11 afp.mp.
12 (unconjugated estriol or unconjugated oestriol).mp.
13 ue3.mp.
14 inhibin$.mp.
15 ultrasound.mp.
16 amniocentesis/
17 chorion$ vill$ sampling.mp.
18 Chorionic Villi-Sampling/
19 nasal bone.mp.
20 tricuspid regurgitation.mp.
21 ductus venosus.mp.
22 marker$.mp.
23 screen$.mp.
24 detect$.mp.
25 accura$.mp.
26 predict$.mp.
27 ROC.mp.
28 ROC curve/
29 AUC.mp.
30 "area under curve".mp.

31 exp false negative reactions/ or exp false positive reactions/
32 (false positive$ or false negative$).mp.
33 likelihood ratio$.mp.
34 sensitiv$.mp.
35 specific$.mp.
36 diagnos$.ti,ab.
37 "reproducibility of results".mp.
38 reference value$.mp.
39 reference standard$.mp.
40 exp Down Syndrome/
41 downs syndrome.mp.
42 down syndrome.mp.
43 trisomy 21.mp.
44 aneuploidy.mp.
45 mosaicism.mp.
46 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).mp.
47 or/1-39
48 or/40-45
49 47 and 48 and 46
****

Search terms and instructions for Biosis

The following search terms were entered separately in standard search box (select 'Titles/subject/abstract' from the drop-down box on the right of the search box).

1. "reference standard*"
2. "reference value*"
3. "reproducibility of results"
4. diagnos*
5. sensitiv*
6. specific*
7. "likelihood ratio*"
8. "false negative*
9. "false positive"
10. "area under curve"
11. ROC
12. AUC
13. predict*
14. detect*
15. marker*
16. screen*
17. accura*
18. "ductus venosus"
19. "nasal bone"
20. "tricuspid regurgitation"
21. "chorion* vill* sampling"
22. amniocentesis
23. ultrasound
24. inhibin*
25. "unconjugaed oestriol"
26. "unconjugated estriol"
27. afp
28. "alpha fetoprotein*"

29. alphafetoprotein*
30. " bhcg"
31. "human chorionic gonadotrophin"
32. "papp a"
33. "pregnancy associated plasma protein"
34. "nuchal translucency"
35. foetal
36. fetal
37. foetus
38. foetal
39. prenatal*
40. antenatal*
41. pregnan*
42. maternal*
43. "trisomy 21"
44. mosaicism
45. "down* syndrome"

The search then used the history function to combine terms:
1-34 - combine using OR
35 - 42 - combine using OR
43 - 45 - combine using OR
The three sets were combined using AND

The combined search strategy had the form
(((((((al: "trisomy 21") or (al: (mosaicism))) or (al: "down* syndrome"))) and ((((((((((((((((((((((((((((((((al: "reference standard*") or (al: "reference value*")) or (al: "reproducibility of results")) or (al: (diagnos*))) or (al: (specific*))) or (al: (sensitiv*))) or (al: "likelihood ratio*")) or (al: "false negative*")) or (al: "false positive*")) or (al: "area under curve")) or (al: (auc))) or (al: (roc))) or (al: (predict*))) or (al: (accura*))) or (al: (detect*))) or (al: (screen*))) or (al: (marker*))) or (al: "ductus venosus")) or (al: "tricuspid regurgitation")) or (al: "nasal bone")) or (al: "chorion* vill* sampling")) or (al: (amniocentesis))) or (al: (ultrasound))) or (al: (inhibin*))) or (al: "unconjugated oestriol")) or (al: "unconjugated estriol")) or (al: (afp))) or (al: "alpha feto protein*")) or (al: "alpha fetoprotein*")) or (al: "b hcg")) or (al: "human chorionic gonadotropin")) or (al: "papp a")) or (al: "pregnancy associated plasma protein")) or (al: "nuchal translucency"))))) and ((((((((((al: (foetal)) or (al: (fetal))) or (al: (foetus))) or (al: (fetus))) or (al: (pregnan*))) or (al: (trimester*))) or (al: (prenatal*))) or (al: (antenatal*))))))

****
The Database of Abstracts of Reviews of Effectiveness (DARE), National Research Register and Health Services Research Projects in Progress database
:
1. Down syndrome (MeSH)
2. down* next syndrome
3. trisomy
4. aneuploidy
5. mosaicism
6. OR/ 1-5

****
MEDION (http://www.mediondatabase.nl/)

ICPC code for pregnancy - 'W'.
****
The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine - download the database to a .pdf file and search for the following terms separately:

Down
Trisomy
Aneuploidy
Pregnant
Pregnancy
Pregnancies
Mosaicism
****

Appendix 2. Glossary of terms (adapted in part from the UK National Screening Committee Glossary)

Abnormal ductus venosus flow velocity	The ductus venosus is a vessel in the fetus which allows oxygenated blood from the placenta to bypass the fetal liver and flow straight to the heart. In conditions such as Down's syndrome the pressure in this vessel can be abnormally high
Absent nasal bone	Absence of the bone that forms the bridge of the nose, which may be detected at ultrasound scan during early pregnancy
Affected individuals	Those individuals who are affected by the disorder for which they are being screened
Amniocentesis	Amniocentesis is an invasive procedure which involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks' gestation
Chorionic villus sampling (CVS)	Chorionic villus sampling involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks' gestation
Combined test	First trimester test (up to 13 + 6 weeks of pregnancy) based on combining nuchal translucency (NT) measurement with free beta-hCG, pregnancy-associated plasma protein A (PAPP-A) and the woman's age
Diagnostic accuracy	The amount of agreement between the information from the index test and the reference standard (see below)
Diagnostic test	A definitive test, performed after a positive screening test result that gives a diagnosis (i.e. yes or no)?
Double test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG ß either free beta-hCG or total hCG), together with the woman's age
First trimester	Pregnancy from conception up to 13 weeks and 6 days.
Iatrogenic	A disease or condition in a patient occurring as a result of treatment
Index test	A test or group of tests being evaluated in a systematic review

(*Continued*)

Integrated test	Measurements performed at different times of pregnancy combined into a single test result. Unless otherwise specified, 'integrated test' refers to the combination of nuchal translucency measurement and PAPP-A in the first trimester, with the quadruple test (see below) in the second
Mosaicism	This is a condition in which person has some cells containing a normal number of chromosomes, and some containing an abnormal number. The more abnormal cells there are, the greater the effect
Multiple of the median (MOM)	The serum test concentration for a pregnant woman divided by the average (median) for unaffected pregnancies in a defined population at the same stage of pregnancy
Quadruple test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of AFP, uE3, free beta-hCG (or total hCG), and inhibin-A together with the woman's age
Reference Standard	The best available method for establishing the presence or absence of the target disease or condition
Second trimester	Pregnancy from 14 weeks to 28 weeks' gestation. Note that for the purposes of this Cochrane review, second trimester testing refers to the period of 14 to 24 weeks' gestation
Tricuspid regurgitation	Leakiness of or backflow of blood through the tricuspid valve of the heart. The tricuspid valve separates the upper and lower chambers of the right side of the heart
Triple test	Second trimester test (from 14 up to 24 weeks of pregnancy) based on the measurement of AFP, unconjugated oestriol (uE3), and hCG (either total hCG or free beta-hCG) together with the woman's age
Trisomy	The presence of an extra chromosome resulting in three copies of a particular chromosome instead of the normal two
Translocation	Part of one chromosome is broken off and attached to another chromosome. This does not usually cause the individual any problems as they have a normal amount of chromosomes, but in an abnormal arrangement. It can be passed on as an extra chromosome to offspring, resulting in conditions such as Down's syndrome

Appendix 3. QUADAS questionnaire

QUADAS criteria included the following 10 questions.

1. Was the spectrum of women representative of the women who will receive the test in practice? (criteria met if the sample was selected from a wide range of childbearing ages, or selected from a specified 'high risk' group such as over 35s, family history of Down's Syndrome, multiple pregnancy or diabetes mellitus, provided all affected and unaffected fetuses included that could be tested at the time point when the screening test would be applied; criteria not met if the sample taken from a select or unrepresentative group of women (i.e. private practice), was an atypical screening population or recruited at a later time point when selection could be affected by selective fetal loss)

2. Is the reference standard likely to correctly classify the target condition? (amniocentesis, chorionic villus sampling, postnatal karyotyping, miscarriage with cytogenetic testing of the fetus, a phenotypically normal baby or birth registers are all regarded as meeting this criteria)

3. Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?
4. Did women receive the same reference standard regardless of the index test result?
5. Was the reference standard independent of the index test result (i.e. the index test did not form part of the reference standard)?
6. Were the index test results interpreted without knowledge of the results of the reference standard?
7. Were the reference standard results interpreted without knowledge of the results of the index test?
8. Were the same clinical data (i.e. maternal age and weight, ethnic origin, gestational age) available when test results were interpreted as would be available when the test is used in practice?
9. Were uninterpretable/intermediate test results reported?
10. Were withdrawals from the study explained?

CONTRIBUTIONS OF AUTHORS

KA undertook the searches, applied eligibility criteria, extracted and entered data and wrote the first and second draft of the review.

ZA applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

JD supervised and planned the review, checked data extraction, supervised statistical analyses and wrote the second draft of the review.

JN applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

BG checked data extraction and undertook statistical analyses.

MP applied eligibility criteria, extracted and entered data for the updated literature search, and entered characteristics of studies information.

YT checked data extraction, undertook statistical analyses, contributed to the first draft of the review, and approved the final draft of the review.

DECLARATIONS OF INTEREST

KA: None known.

ZA: None known.

JD: None known.

JN: None known.

BG: None known.

MP: None known.

YT: None known.

SOURCES OF SUPPORT

Internal sources

- University of Birmingham, UK.

Funding of the research time of JD and BG

External sources

- NIHR Health Technology Assessment Programme, UK.

Project grant - need to have reference number etc. Jim/Zarko can you add please?

- NIHR Health Technology Assessment Programme, UK.

Funding for the Cochrane Reviews of Diagnostic Test Accuracy Support Unit, based at the University of Birmingham (JD).

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The protocol intended to investigate several additional outcomes downstream from test accuracy, should they be reported in the test accuracy studies. When we attempted to extract this information however, it was found to be available in very few studies, and where such information was found it was difficult to extract meaningful data to allow for comparison between studies, as data were not reported in a universal manner. In several studies such outcomes were estimated rather than measured. Often they were not reported at all. The outcomes stated in the protocol which have not been included are: harms of testing; need for further testing; side effects of test; interventions and side effects; other abnormalities detected by testing; spontaneous miscarriage; miscarriage subsequent to invasive procedure, with or without normal karyotype; fetal karyotype; termination of pregnancy (prior to definitive testing or in a karyotypically normal pregnancy and following confirmation of Down's syndrome or following detection of other chromosomal abnormalities); stillbirth; livebirth of affected and unaffected fetus; uptake of definitive testing by women.

The following refinements to the eligibility criteria were imposed to ensure that the quality of the included literature remained high. We excluded studies that identified fewer than five Down's syndrome pregnancies in their study population. We excluded studies that had less than 80% follow-up of participants.

In addition, the analytical strategy was informed by the volume of tests and studies included, and developed so that we focused on key tests and test combinations by a) only meta-analysed tests that were included in four or more studies or b) showed more than 70% sensitivity for more than 95% specificity. In addition, a requirement that a minimum of 10 studies for a single test was required before subgroup analysis was undertaken. Consequently several possible sources of heterogeneity were not investigated due to lack of data.

NOTES

This is one of a suite of planned systematic diagnostic test reviews planned for prenatal testing for fetal Down's syndrome. The plans for these reviews were described in a generic protocol (Alldred 2010) published in the Cochrane Library in 2010. The five reviews were to be of: first trimester serum tests only; first trimester ultrasound tests alone, and in combination with first trimester serum tests; second trimester serum tests only; first and second trimester serum tests with and without first trimester ultrasound tests; and urine tests. One of these reviews has been published already (Alldred 2012). Diagnostic test reviews are relatively new, and this project has proven much larger, more complex and difficult to complete than had been anticipated. Whilst not fulfilling the usual Cochrane up-to-date criteria (the electronic search was done in 2011), this review is published because it provides historical context in what is a rapidly-changing field, and because it is unlikely to ever be repeated.